University of Reading

The effect of priming seeds with plant growth regulators on the growth and development of rice

For the degree of Doctor of Philosophy

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Declaration of original authorship:

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledged.

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Abstract

Water scarcity is a major concern for agricultural production worldwide. The staple crop, rice, is one of the most inefficient crops in its consumption of water using 30% of the world's freshwater resources. Therefore, research for appropriate solutions to overcome this problem is necessary. Plant growth regulators (PGRs) could be one of these solutions, stimulating cell division and elongation in the root system during early plant growth, which can help seedlings to establish a strong root system. Increasing the root length of plants can allow them to access water at depth for a longer period and increase their tolerance to drought conditions. Seedling growth assays were conducted to investigate the effect of soaking seeds for 24 hours in different PGRs. Rice genotype IR64 was used to investigate the optimal time for seedling growth assays and to quantify the effects of soaking seeds in six PGRs at four different concentrations on seedling growth. Subsequently, twenty rice genotypes were investigated with one selected concentration and four PGRs compared with water (control) to highlight the variation in root and shoot trait responses. Genotype GHRAIBA was selected to investigate the effect of 1000 µM gibberellin (GA3) seed treatment on growth and development under drought stress. Results confirmed there was a significant response to GA3 treatment during the early growth stages. Although, this effect was not observed 40 days after sowing (DAS), at maturity (139 DAS) plants whose seeds were treated with 1000 µM GA3 had significantly ($P<0.001$) increased root fresh and dry weight. The transcriptional analysis of seedlings treated with GA3 showed only *Os04g0612500* was upregulated. This gene is similar to a proline-rich protein which is involved in protein protection under stress. These results could be used to develop practical approaches to improve drought tolerance in rice crops.

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Contents

List of Figures:

List of Tables:

1. Introduction (Literature Review)

1.1. Background

1.2. Global water availability

Water plays a crucial role in peoples' lives. It is considered the main component in every organism. Limited water availability will be a major dilemma facing the entire world in the next century (Figure 1.1). While the estimated total volume of global water is 1,386,000,000 km^3, 97.5% is classified as saltwater and 2.5% is classified as freshwater. Glaciers and ground water make up 98.76% of this freshwater, meaning that only 1.2% of the freshwater is accessible for agriculture, industrial and domestic use (Programme, 2009, Connor *et al.*, 2017). One commonly used definition of water scarcity in the societies is when domestic water usage is below 1000 m^3 $person^{-1}$ $year^{-1}$. In areas that suffer from arid and semi-arid environments, the amount of available water is often below 500 m^3 $person^{-1}$ $year^{-1}$ (Pereira *et al.*, 2009). Fader *et al.* (2016) indicates that, the agriculture at the Mediterranean basin is likely to be increasingly affected by water scarcity as a consequence of climate change. When CO_2 concentration increase combined with increasing global warming from 2°C to 5°C, this will result in, increase irrigation water requirements by 18%. In addition, the increasing use of aquifers in arid and semi-arid environments are highly likely to lead to exhaustion of all sub-soil water (Taylor *et al.*, 2013). As a consequence of water depletion by population, agriculture and industrial, global fresh water consumption is expected to double over the next decade, increasing to 3,800 km^3 $year^{-1}$ by 2025 (Jury & Vaux, 2005). Globally, the agriculture sector withdraws more water than any other sector. However, there is some variation between the continents, for example Asia and Africa withdraw water for agriculture use almost four times higher than Europe (Figure 1.1) (Morison *et al.*, 2008).

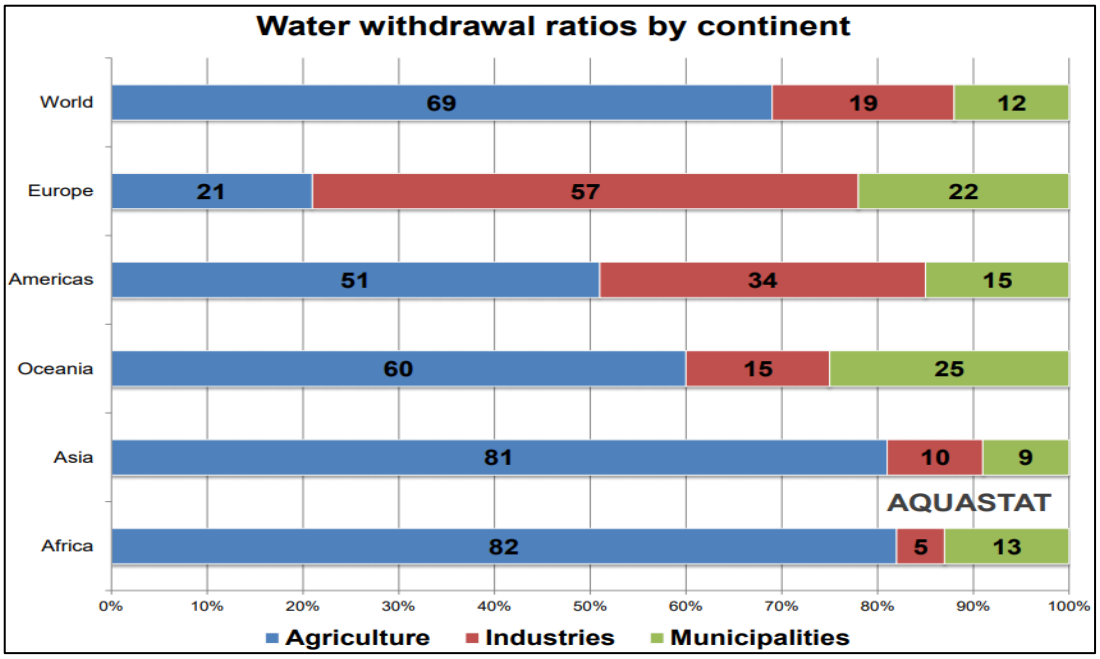


Figure 1.1. Water withdrawals for agriculture, industry and households in different world regions. FAO (2015).

1.3. Risks to water availability

Climate change may impact on water availability in certain areas of the world (Elliott *et al.*, 2014). Climate change is thought to be a consequence of anthropogenic greenhouse gas emissions into the earth's atmosphere, reducing the release of the sun's energy from the planet's surface and increasing temperatures (Ramanathan & Feng, 2009). This process has an impact on all natural systems on Earth, including accelerating glacier melting, floods, high temperatures, drought and shifting seasons (Nelson *et al.*, 2009, Reynolds, 2010, Lobell & Gourdji, 2012). Climate change will have significant impacts on agriculture through changes in rainfall patterns (drought or flooding), higher temperatures, changes in seasonality etc. (Gornall *et al.*, 2010). Since, agricultural activities withdraw 69% freshwater worldwide, reduced freshwater availability poses a significant risk, especially in Asia and Africa, where agricultural water use is high (Figure 1.1). As an increasing effect of climate changes, drought has become a serious threat for global agriculture and crop production in particular (Mohanty *et al.*, 2016).

These changing weather patterns will not only increase pressures on freshwater resources for their use in the irrigation of agricultural production systems but might also reduce water for rainfed systems. The increasing global population will also place continuing demands on freshwater use, directly through domestic use and indirectly through their increasing need for food through agricultural production systems. This will create a nexus of pressures with food, energy and water (Ringler *et al.*, 2013).

1.4. Water use in agriculture

Globally, the agriculture sector withdraws more water than any other sector (Morison *et al.*, 2008, Cosgrove & Rijsberman, 2014), on average consuming 69% of the freshwater resources (Figure 1.1). The projected water withdrawals by the agricultural sector in 2025 are estimated to be almost 90% of the total water consumption in the world (Programme, 2009). Furthermore, continuing withdrawals of freshwater without management of water resources will lead to 60% or more of world suffering from water scarcity (Cosgrove & Rijsberman, 2014). Therefore, freshwater supplies need to be used more sustainability. Researchers and growers around the world recognize that water is the single most important abiotic factor limiting crop productivity (Vadez *et al.*, 2013).

1.5. Crop responses to drought

Water scarcity significantly affects crop production (Lambers *et al.*, 2008, Rich & Watt, 2013, Cosgrove & Rijsberman, 2014). It has a range of morphological to molecular effects during plant growth that ultimately lead to a reduction in crop production (Farooq *et al.*, 2009c). Water scarcity or drought can reduce plant growth by affecting photosynthesis through stomatal closure. This may reduce the

availability of water for photosynthesis reactions, reducing carbon fixation and growth (Pinheiro & Chaves, 2010).

Drought can be classified into two patterns: (i) terminal drought or long-term period of water deficit with no rain or other precipitation and (ii) intermittent drought which can occur at any growth stage of plant growth (Tsubo *et al.*, 2006).

Water limitation impacts firstly the plant leaves and actively growing regions such as the shoot meristem, new leaves and new tillers. Water limitation also reduces stomatal and mesophyll conductance (Farooq *et al.*, 2009c). Drought often coincides with high irradiance and high temperature stresses (Lambers *et al.*, 2008). Consequently, photoinhibition can also limit plant growth. Reduced water uptake lowers the water potential of plant cells, which increases the concentrations of solutes in the cytosol and extracellular matrices. Consequently, cells are unable to grow, leading to growth inhibition and reproductive failure (Lisar *et al.*, 2012). The duration and severity of the water deficit and whether it occurs through the vegetative growth stages or reproduction stages can determine, how significant crop productivity is affected (Nuccio *et al.*, 2018). There is still a knowledge gap that needs to be filled about the genetic regulation of crop responses to drought (Barker *et al.*, 2005, Ribaut & Ragot, 2006, Yang *et al.*, 2010, Joshi *et al.*, 2016).

1.6. Strategies for water management in agriculture

The most important issue related to water scarcity is water management (Boyer, 1982). There are two strategies that can significantly help to manage water usage in agriculture. One of them is limiting the expansion of irrigated agriculture and the other is increasing water use efficiency in both water supply network and by crops through breeding. These two mechanisms have the potential to save approximately 35% of global freshwater resources (Jaleel *et al.*, 2009, Cosgrove & Rijsberman,

2014). Therefore, innovative research is required to improve agricultural water use efficiency (Pereira *et al.*, 2002, Greenwood *et al.*, 2010), through more efficient use of available water, breeding new crop varieties that require less water or are better able to access water through their root systems, and minimising wastage on farm (Evans & Sadler, 2008). Since plant root systems are critical for efficient water uptake, improving the root system to acquire more water can be achieved through different techniques such as breeding, transgenic approaches and chemical enhancement of the root system by using plant growth regulators (PGRs) (Ferguson & Lessenger, 2006). Under environment restrictions such as drought, salinity and the poor soil resources in the upper parts of the soil, low yields and food insecurity are most likely to occur. Water availability during plant growth stages is considered one of the most crucial elements for plant growth and development, in parallel with using plant fertilizers. Consequently, improving crop productivity through developing a strong root system with improved drought tolerance could be the key factor to overcome drought restriction (Lynch, 2007, Lynch, 2013).

1.7. Plant growth regulators

Plant growth regulators (PGRs) are chemicals produced by plants to regulate development and adaptations to stress or are synthesised artificially to control plant growth (Santner *et al.*, 2009, Van Ha *et al.*, 2014, Fahad *et al.*, 2015). PGRs are produced at low concentrations in plants, where they can functionally affect either the cells in which they are synthesised or other parts of the plant after they have been transported. Plant responses to PGRs are mediated through cell receptors that trigger transcriptional changes that may alter protein degradation, hormone signalling and/or hormone interactions (Santner *et al.*, 2009). PGRs include a number of different compounds including gibberellins, cytokinins, auxins, ethylene, abscisic acid, phenolics and alkaloids (Table 1.1). Shoot development can be

positively affected by cytokinins or gibberellin; conversely, the root system is negatively affected by cytokinins (Werner *et al.*, 2001, Werner *et al.*, 2003, Saito *et al.*, 2006). PGRs can regulate root responses to stress by sending different signals to all plant compartments. For example, under drought stress, the plant hormone abscisic acid (ABA) can be triggered which results in generation of chemical signal(s) for shoot and root to regulate water uptake, stomatal closure and restriction of cell growth (Wilkinson & Davies, 2002, Sharp *et al.*, 2004, Schachtman & Goodger, 2008). Soaking Arabidopsis seeds in auxin defers seed germination in a saline environment by upregulating the IAA30 gene. However, there was no effect of soaking Arabidopsis seeds in 10 µM IAA on seed germination under high salinity conditions of 150 mM NaCl (Park *et al.*, 2011).

Sheteiwy *et al.* (2018), stated that, using 2.5 mM methyl jasmonate (MeJA) induced plant responses to osmotic stress (imposed with polyethylene glycol (PEG) 6000 30 g/L) compared with 5 mM MeJA. Seeds soaked in 2.5 mM MeJA had improved germination and vigour compared with seeds soaked in 5 mM MeJA, which reduced both parameters. Results also showed that using PEG to reduce water availability negatively affected seedling root length, shoot length, fresh weight and dry weight. However, using 2.5 mM MeJA significantly improved these seedling parameters (Sheteiwy *et al.*, 2018). Water availability also enhances the biosynthesis and transport of auxin, which is responsible for lateral root initiation and also necessary for effective hydropatterning, which is the development and initiation of lateral roots from the primary root and aerenchyma formation. However, under drought stress condition lateral root initiation is restricted and ABA PGR triggered. (Werner *et al.*, 2010, Bao *et al.*, 2014).

Table 1.1. Plant growth regulator class, associated function(s) and practical uses.

Class	Function(s)	Practical uses	Reference
Auxins	Shoot and root elongation, ability of auxin to direct cell division, expansion, and differentiation	Auxin responses are highly context dependent and can involve changes in cell division, cell expansion, maintenance of stem cell niches, ripening and cell fate.	(Vanneste & Friml, 2009, Salehin *et al.*, 2015)
Gibberellins	Stimulate cell division and elongation	Gibberellins (GAs) are a class of diterpenoid acids that regulate many aspects of plant growth and development including seed germination, stem elongation, leaf expansion, and flower and fruit development	(Sun, 2010, Binenbaum *et al.*, 2018)
Cytokinins	Stimulate cell division	Cytokinins play a key role in plant morphology, plant defence and leaf senescence.	(Giron *et al.*, 2013)
Ethylene	Ripening, biotic stress responses such as pathogens	Ethylene is a gas that acts as a regulator of plant growth, including the promotion of root hair elongation and the regulation of uniform ripening in fruit and vegetables.	(Abeles *et al.*, 1992, Dubois *et al.*, 2018)
Synthetic growth inhibitors	Stops or slows growth	Can inhibit endogenous PGR production of perception to alter growth.	Han *et al.*, 2009

Abscisic acid	Stress tolerance	Promotes stomata closure by rapidly altering ion fluxes in guard cells.	(Levchenko *et al.*, 2005)
Strigolactones	Tiller and shoot branching inhibitor	Strigolactones are a group of terpenoid lactones that act as an inhibitor of shoot branching and promote associations with arbuscular mycorrhiza.	(Arite *et al.*, 2009, Umehara *et al.*, 2010, Zha *et al.*, 2019)

1.7.1 Abscisic acid (ABA)

ABA was first identified in the 1960s, in mesophytic plants under water-stress (Hsiao, 1973, Davies, 2010). Since that time, many studies refer to ABA as a first line of defence for plants under drought stress. In response to drought stress, ABA concentrations increase, and activates two responses. The first is to reduce or close their aperture of stomata and the second is to induce stress-related transcription of genes (Yang *et al.*, 2002, Yamaguchi-Shinozaki & Shinozaki, 2005, Fujii & Zhu, 2009, Cutler *et al.*, 2010, Hubbard *et al.*, 2010). Although, ABA is well known as a stress-related PGR, it also involved with development and growth processes (Cutler *et al.*, 2010).

ABA concentration in the tissue and the sensitivity of the tissue can determine plant responses to this PGR. Likewise, biosynthesis, catabolism, compartmentation, and transport processes control ABA concentration within plant tissues (Taiz & Zeiger, 2006). The ABA biosynthesis starts from isopentenyl pyrophosphate (IPP) (Ye *et al.*, 2012). ABA biosynthesis occurs in chloroplasts and other plastids and then catalysed by a zeaxanthin epoxidase enzyme (Taiz & Zeiger, 2006). During seed development, in the maturation stage, ABA is necessary to control switching

between embryogenesis and germination stages. When seed maturation begins, ABA accumulation increases with storage accumulation and plays a crucial role in embryo development for angiosperms (Davies, 2010, Finkelstein, 2013, Dekkers *et al.*, 2016). Both xylem and the phloem can be used to transfer ABA. However, phloem appears to be more involved in ABA transport (Kudoyarova *et al.*, 2011). In a study conducted by Kudoyarova *et al.* (2011), they demonstrate that, ABA controls water relations in wheat under stress conditions. Wheat seedlings were exposed to an increase in air temperature by 3°C from 22 to 25°C with 60% relative air humidity. Results suggest that ABA concentration increased in the root system in contrast to shoots. This indicates that redistributed ABA in response to air warming stress, is used to regulate water relations, not just under drought stress (Kudoyarova *et al.*, 2011).

1.7.2 Gibberellins

Gibberellins (GA) were first discovered in the 19th century. First isolated from a rice pathogenic fungus, this fungus caused abnormal growth of rice seedlings (Davies, 2010, Hedden & Sponsel, 2015). GA consist of 126 different types of tetracyclic diteroinoid compounds synthesised by higher plants, bacteria and fungi (Hedden *et al.*, 2015). In plants the GA-biosynthetic pathway occurs over three sub-cellular locations, starting in plastids, then the endoplasmic reticulum and finishing in the cytosol (Figure 1.2). The biosynthesis pathway starts in the plastids, where trans-geranylgeranyl diphosphate (GGDP) is converted to ent-copalyl diphosphate (CDP), then to the tetracyclic hydrocarbon ent-kaurene by CDP synthase (CPS) and ent-kaurene synthase (KS) respectively (Figure 1.2). Following a series of oxidations by Cytochrome P450 monooxygenases, ent-kaureneoxidase (KO) and ent-kaurenoic acid oxidase (KAO) a final bioactive form of GA12 is produced (Sakamoto *et al.*, 2004, Yamauchi *et al.*, 2004, Davies, 2010). The final bioactivation steps occur in

cytoplasm through either the early-13-hydroxylation pathway or the non-13-hydroxylation pathway, followed by oxidation to a range of bioactive GAs (Figure 1.2). Manipulation of these enzymes can result in reduced GA synthesis. This has been exploited in rice (and other crops), where mutations in GA20ox genes, related to semi-dwarf mutations at the *sd1* locus, have been used to obtain dwarf genotypes of rice. This mutation works on limiting GA production, resulting in semi-dwarf rice genotypes, which are more tolerant to damage caused by wind and rain, and allocate more resources to their grains (Spielmeyer *et al.*, 2002, Sakamoto *et al.*, 2003, Sakamoto *et al.*, 2004).

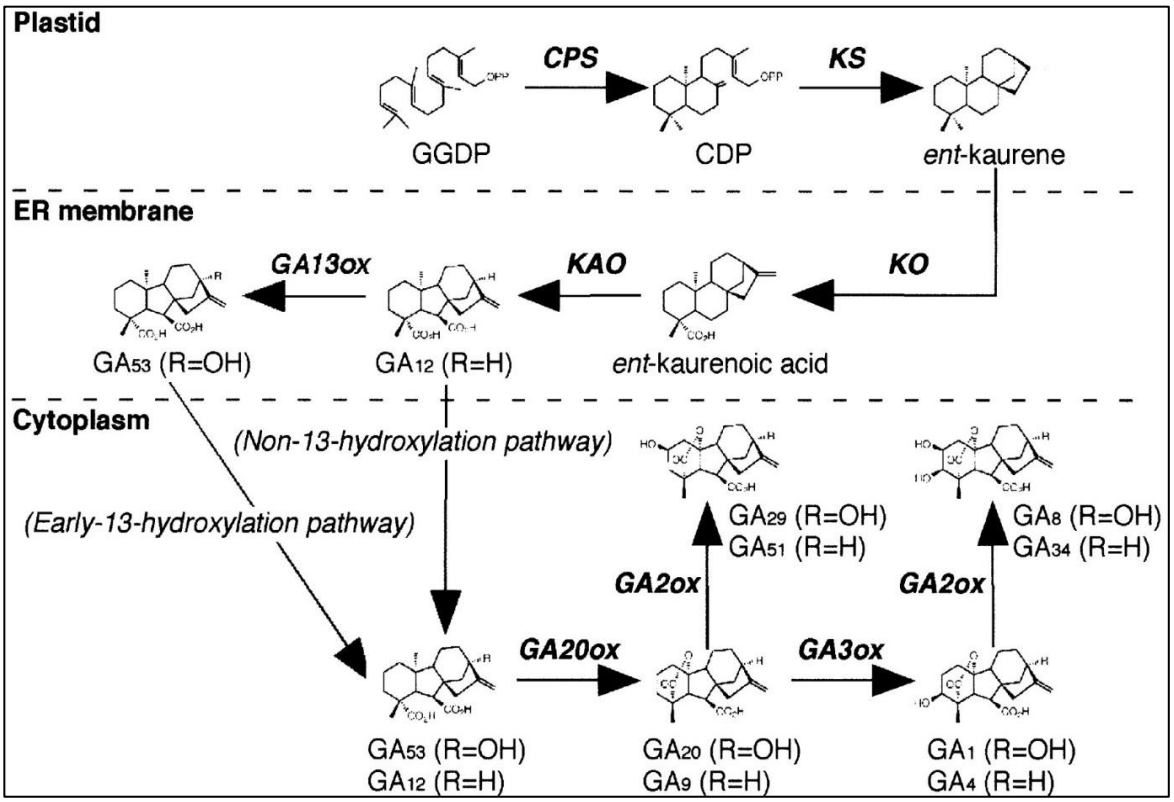


Figure 1.2. Gibberellins biosynthesis and activation pathway, dotted lines refer to phases. (GGDP) refers to geranylgeranyl diphosphate, (CDP) refers to ent-copalyl diphosphate. Steps are catalysed by ent-copalyl diphosphate synthase (CPS), ent-kaurene synthase (KS), ent-kaurenoic oxidase (KO), ent-kaurenoic acid oxidase (KAO). Image from Sakamoto et al, 2004.

Gibberellins are well known for their effect on the shoot system by stimulating elongation. In an attempt to identify GA transporters in Arabidopsis, by using the RNA which extracted from two weeks old seedlings, SWEET proteins, two mutants AtSWEET13 and AtSWEET14 has been suggested to modify GA response in Arabidopsis, through altering GA uptake by cells(Kanno *et al.*, 2016). However, there is still a lack of information about root growth regulation by GAs. This is because the full transportation mechanism of GAs is still incomplete (Binenbaum *et al.*, 2018). Recent micrografting and biochemical analysis in Arabidopsis suggests that the GA precursor, GA12, is mobile in the xylem and phloem tissue and may allow long distance signalling of GA signals (Tanimoto, 2005, Regnault *et al.*, 2016). In Arabidopsis, GA enhances germination when the seeds are exposed to exogenous GA. The expression of a number of GA-regulated genes increased before seed germination, including genes responsible for synthesis, transport, and signalling of other hormones (Ogawa *et al.*, 2003).

Not all gibberellin forms are active, only GA1 was suggested to be the active form of gibberellin. GA1 was observed to be presented in tall plants compared to dwarf plants. Thus, the GA20 the non-active forms of GAs converted to GA1 by activating Mendel's tallness gene. This gene then coding GA 3β-hydroxylase (3-oxidase) enzyme to catalyse the conversion (Sakamoto *et al.*, 2004). Restricted plant growth under abiotic stress is highly linked with GA levels reduction. This indicates and clarifies the GA role in response to abiotic stress. One of the suggested mechanisms for GA in response of abiotic stress is that, GA works synergistically with Jasmonate (JA) hormone through the GA-signalling molecule DELLA interacting with some receptors during the pathway for the stress hormone jasmonic acid.

This may suggest, there are probably another signalling pathways in which GA works synergistically or antagonistically in response of stress. In response to drought condition and to reveal the GA role under drought condition, wild type and five different mutant lines of Arabidopsis thaliana were exposed to drought. These mutants were 35S:GA20ox (high GA content), ga2ox (high GA content), ga20ox1/2 (GA content reduced by demolishing of AtGA20ox1 and AtGA20ox2), ga3ox1/2 (reduced GA content) and ga20ox1/2/3 (very low GA content). The results for the plant shoots indicate that, mutants with reduced GA were more resistance to drought than the high content GA (Colebrook *et al.*, 2014). Using the meta-analysis, the GA biosynthesis transcriptional responses and deactivation genes on the wild type of emmer, with the effect of drought and osmotic stress was explained. The results referred that, GA2ox expression in root was decreased (Hruz *et al.*, 2008, Krugman *et al.*, 2011). This resulted in focusing metabolism outcome to counter the stress by changing the plant growth rate (Skirycz & Inzé, 2010). However, not many papers investigate the effect of drought stress on the GA metabolism, therefore this relationship is still not fully understood.

1.7.3 Auxins

Auxins are a class of PGR that regulate plant development and adaptations to environmental cues (Blakeslee *et al.*, 2005). Auxin biosynthesis occurs in meristematic regions of shoots and roots (Ljung *et al.*, 2001, Ljung *et al.*, 2002). Two auxin biosynthesis pathways have been suggested, one of them starting from the amino acid, tryptophan, the other being the tryptophan-independent pathway. The tryptophan pathway begins by converting tryptophan to tryptamine which is a monoamine alkaloid and to N-hydroxytryptamine, ending with indole-3-acetaldoxime (Figure 1.3). Through this process, YUCCA genes play a crucial role

in converting tryptamine to N-hydroxytryptamine (Yamamoto *et al.*, 2007, Davies, 2010, Mano & Nemoto, 2012). The tryptophan-independent pathway could be considered as a supportive pathway for the dependent pathway when higher levels of auxin are required. This pathway starts from converting indole-3-glucosinol phosphate (IGP) directly to indole-3 acetic acid (IAA) (Zazimalova & Napier, 2003).

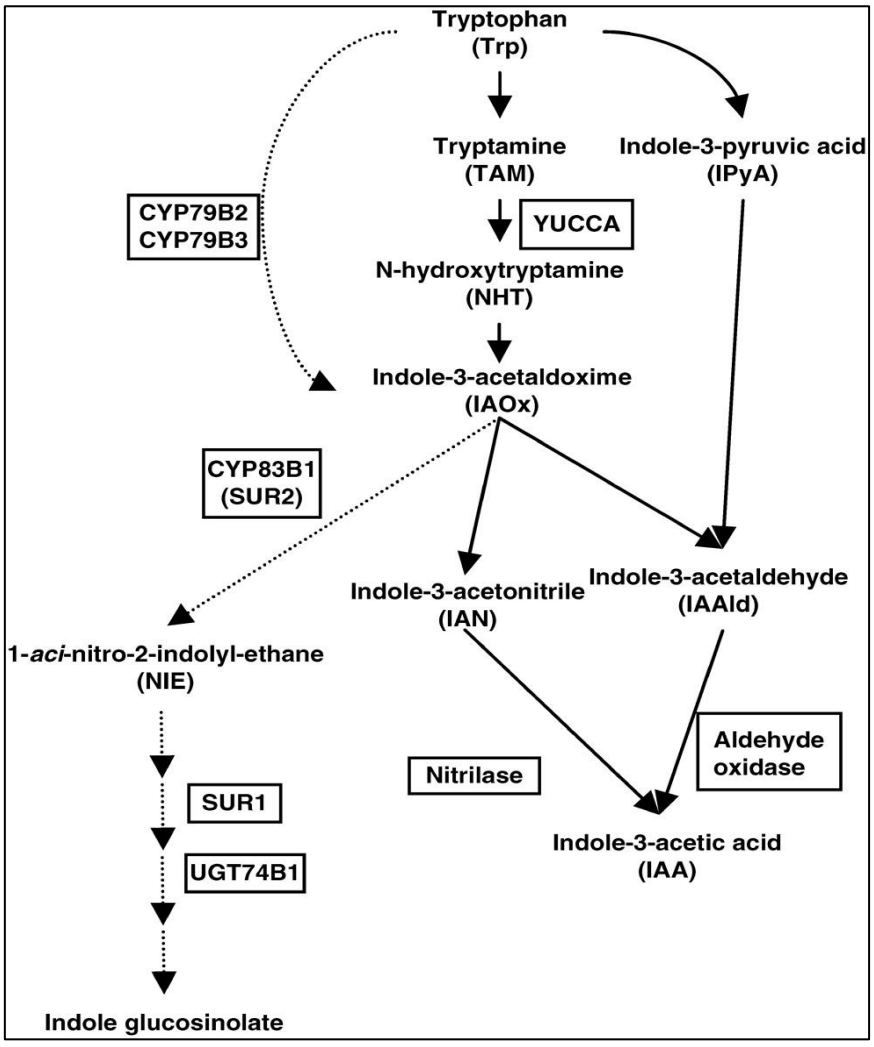


Figure 1.3. Auxin biosynthesis and activation through Trp-dependent pathway. Boxes indicate to genes or mutants linked to specific enzymatic steps. Image from Yamamoto *et al.,* 2007.

Auxin is present in the shoot and root system and is transported via IAA-carrier proteins like AUX1, PINs and MDRs to support the transport of auxin through plant tissues. Auxin concentrations in the root system depend on influx and efflux from the cells, with most auxin influx coming from the shoot system, since auxin biosynthesis is not significant in the root system (Tanimoto, 2005, Geisler *et al.*,

2005, Peer *et al.*, 2011). Influx of auxin into the cell depends on the auxin form, thus 1-naphthaleneacetic acid (NAA), a synthetic auxin analogue, has the advantage of greater physicochemical penetration through cell membranes compared with IAA (Yamamoto & Yamamoto, 1998, Marchant *et al.*, 2002). Furthermore, NAA controls lateral root formation (Overvoorde *et al.*, 2010). Two steps have been identified for auxin in stimulating lateral root initiation, starting with stimulation of mitosis for parenchyma or sclerenchyma cells to start preparing for primordium development. This step is regulated by auxin concentration through the *ALF1* gene, regulating the number of lateral roots. The second step is maintaining the auxin concentration to continue to support primordium formation and this can be either from increasing the auxin flow or starting local production (Celenza *et al.*, 1995). Both stages are thought to be controlled by AUX1 proteins (Péret *et al.*, 2009). For example, in rice, 7-day-old *OsAUX1* mutant seedlings exhibited fewer lateral roots emerging compared with wild-type rice plants. However, *OsAUX1* expression could be increased by adding 0.01 µM exogenous NAA to rice seedlings. This treatment altered the phenotype of defective *osaux1-1* mutant plants, resulting in the mutant to have a WT phenotype (Zhao *et al.*, 2015).

Using different auxin concentrations could be the key point to understand the auxin function in the root system (Wilkins, 1969). In contrast to NAA, IAA can have a negative impact on root elongation at a range of concentrations (Scott, 1972, Feldman, 1984, Pilet, 2002). Tanimoto (2005) suggested that promoting or inhibiting root and shoot growth depended on using the right concentration of auxin, which was 10^{-9} M for roots and 10^{-5} M for shoots.

1.7.4 Cytokinins

Cytokinins have two biosynthesis pathways, one beginning with the degradation of tRNA and the other beginning with the transfer of cis-zeatin riboside monophosphate (DMAPP) and AMP to isopentenyladenine riboside (iPRMP) (Figure 1.4) (Werner & Schmülling, 2009, Davies, 2010, Frébort *et al.*, 2011). Cytokinin has a controlling role in plant growth and development through a two-component signalling network involving histidine kinases and response regulators (Tran *et al.*, 2010). According to Werner *et al.* (2010), enhancing cytokinin degradation in *Arabidopsis thaliana* and tobacco transgenic plants induced elongation of the primary root, root branching, and root biomass formation and root-to-shoot ratio by 60%. Consequently, using PGRs for increasing the root length of rice plants may allow them to access water at depth for a longer period of time and increase their tolerance to drought conditions (Malamy, 2005). Although cytokinins have been shown to have a growth promoting effect when used with wheat and Barley, benzylaminopurine (BA), which is one form of cytokinin, has been shown to

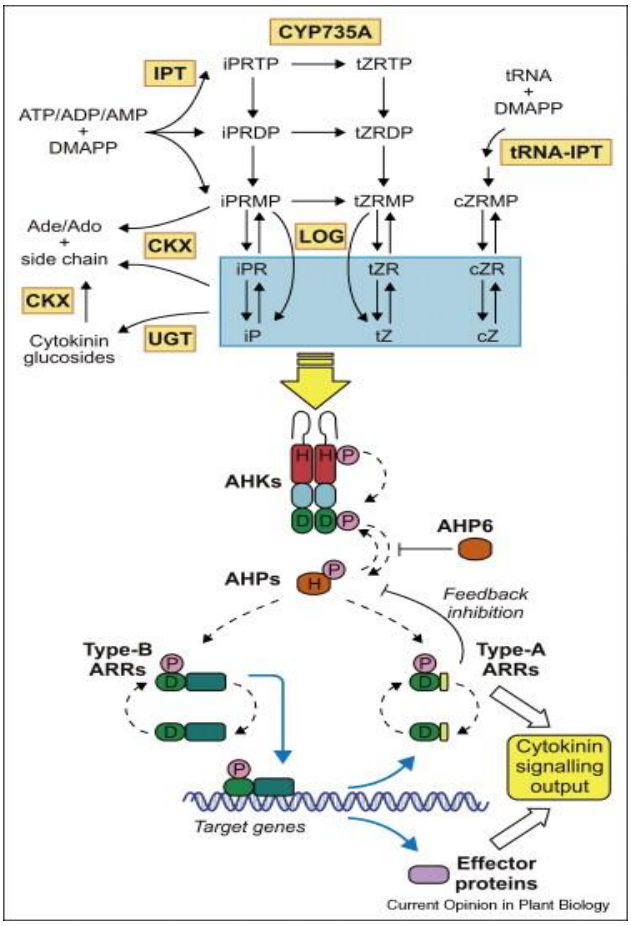


Figure 1.4. Cytokinin biosynthesis and activation pathway. Starting with adenosine phosphate-isopentenyltransferases (IPTs) to tZ-nucleotides through iP-nucleotides controlled by monooxygenases (CYP735As). Blue box highlights cytokinins which are biologically active. (AHKs) indicates histidine kinase receptors, (AHPs) indicates histidine phosphotransfer proteins. Image from Werner *et al.,* 2009.

have a negative effect on shoot and root development in rice (Zaochang *et al.*, 2000).

BA was applied at a concentration of 100 µM to the shoots of hydroponically grown rice supplied with either N in the form of NH_4^+ or NO_3^- or an equal mixture of both forms of N. The BA treatment of rice decreased the adventitious root number by 16% to 23% compared with controls (Zaochang *et al.*, 2000).

Combinations of PGRs can also have positive or negative effects on seed germination and seedling emergence (Kucera *et al.*, 2005). Some PGRs can work synergistically such as gibberellins and cytokinins, significantly improving seed germination (Kaur *et al.*, 2015). Conversely, other PGRs could work antagonistically, for example, the negative effect of ABA on the mitotic index of barley cells was reduced by the presence of GA, KIN and BA in the medium (Tabur & Öney, 2012).

1.8. Current practice of seed priming

Seed priming is a pre-germination technique of soaking seeds to moderately hydrated them to activate metabolic processes but without initiating germination (Farooq *et al.*, 2019). Seed priming is a simple and cost-effective approach which has been used for different crops and in different countries. Seed priming has been shown to improve yield, tolerance to biotic and abiotic stresses(Farooq *et al.*, 2019). A number of approaches have been used to prime seeds, including the use of PGRs, polyethylene glycol or salt solutions, tap water (hydropriming), plant growth-promoting bacteria (biopriming), macro or micronutrients (nutripriming) and some plant-based natural extracts (Farooq *et al.*, 2019). Seed priming can vary from conventional to effective seed priming for micronutrient delivery at planting in field. Hydropriming, on-farm priming and seed hardening are simple, economical and environmentally friendly techniques for seeds priming in tap water with or without

aeration (Afzal *et al.*, 2002, Farooq *et al.*, 2006, Di Girolamo & Barbanti, 2012). While, osmopriming, osmohardening, hormonal priming and matripriming are based on soaking seeds in aerated, low-osmotic potential solutions or in aerated solutions of different plant growth regulators to improve water use and the performance of direct-seeded rice (Rehman, 2011, Rehman *et al.*, 2011, Iqbal & Ashraf, 2013, Lutts *et al.*, 2016). The effects of each of these priming treatments on the growth, development and grain yield in different field crops regard to the of PGRs concentration, micronutrients, osmotica and plant based leaf extracts, including soaking or hydration duration have recently been reviewed (Farooq *et al.*, 2019). Phenological events for primed field-sown crops can be accelerated earlier than non-primed crops. Early establishment and enhanced vigour are also seen in the plants of primed seeds which results in better root systems that could better placed to capture more water and nutrient resources under drought stress environments (Farooq *et al.*, 2006). Seed priming has also been shown to produce plants with larger leaf area indices and enhanced canopy duration with greater photo-assimilation compared with non-primed crops (Farooq *et al.*, 2012, Farooq *et al.*, 2011a, Rehman *et al.*, 2017).

1.9. Drought stress and plant transcriptional responses

Improving crop production in general and rice production specifically under increasing demand for food and water scarcity still represents a significant scientific challenge (Kang *et al.*, 2009, Piao *et al.*, 2010, Ray *et al.*, 2015, Zhao *et al.*, 2017). Many studies have examined the plant phenology and root development relationship under drought stress (Nguyen *et al.*, 1997, Babu *et al.*, 2003, Farooq *et al.*, 2009a, Nada *et al.*, 2019).

However, when this relationship is linked with the genetic regulation of these responses it becomes more complex as many root traits are considered quantitative

traits and are controlled by combinations of genes which work synergistically (Price *et al.*, 2002, Campos *et al.*, 2004). To improve plant responses to drought stress, plant growth regulators could be used to help mitigate the effects of drought. Plant responses to abiotic stress conditions through PGRs signals, the rapid in response to their environment can determine a critical requirement for early adapting to severe environment and survival as an organisms (Colebrook *et al.*, 2014).

This plant response through PGRs signalling networks indicates the important role of PGRs in mediating plant defence responses, as PGRs have been described as "watchdogs of stress response". Therefore, understanding the role of individual PGRs and their interactions in response to abiotic stress is the key to engineering stress tolerant crops (Davies, 2010, Verma *et al.*, 2016).

Inhibiting root expansion and root elongation was suggested to be resulted from GA-depletion. Nevertheless, the role of GA in root systems still needs to be clarified. In contrast, GA increases the degradation of DELLA proteins which is capturing the transcription factors and blocking the transcription process from DNA to mRNA (Ueguchi-Tanaka *et al.*, 2007). This process occurs during the germination after the GA released and bond with DELLA proteins via GID1 receptor. Consequently, resulting in degradation DELLA proteins and releases transcription factors to bind to the gene promoter and activate the gene that trigger transcription and translation processes to produce α-amylase enzyme which is a necessary enzyme to start starch hydrolysis. (Shimada *et al.*, 2008, Ubeda-Tomas *et al.*, 2009).

Plants respond to drought stress through a combination of physiological, cellular, and molecular processes. Many of these processes are regulated through changes in the concentration or sensitivity to PGRs (Pandey *et al.*, 2015). At the molecular level, many genes are activated under drought stress conditions, including a number

of transcription factors (TFs) which work to promote the activation of specific genes or inhibit the activation of others. These genes and TFs can significantly affect plant responses to stress (Hu *et al.*, 2006, Guo *et al.*, 2008). To improve drought tolerance in rice and to achieve sustainable agriculture target, drought-responsive transcriptome was comparatively analysed into two rice genotypes.

Nagina 22 (N22) drought-tolerant genotype, versus IR64 drought-susceptible but high-yielding genotype were used in this study (Lenka *et al.*, 2011). Results suggest 77out of 1900 probes were up-regulated in N22 compared to 14 out of 920 probes down-regulated in IR64 when transcriptome comparison conducted under drought stress. Zinc finger motif portion encoded by genes was found to be (46.15%) among the upregulated TFs for N22 genotype (Lenka *et al.*, 2011). Zinc finger protein–encoding genes in response to abiotic stress is identified in rice to expression of stress defence genes through increasing the amount of free proline and soluble sugars (Xu *et al.*, 2008). Rabbani *et al.* (2003) observed that 100% of ABA-inducible genes were also induced under drought stress, which highlights the significant correlation between drought and ABA responses. The protein 9-cis-epoxycarotenoid dioxygenase (NCED) is an important rate limiting step in the production of ABA. When the gene encoding *NCED* was overexpressed in tomato, the roots accumulated a higher concentration ABA. When the overexpressing lines were subjected to drought the ABA accumulation was further enhanced (Thompson *et al.*, 2007). In rice, drought stress inducible genes were classified into two groups functional proteins and regulatory proteins. The first group was classified to work under abiotic stresses, such us key enzymes for osmolyte biosynthesis and water channel proteins. While the second group was thought to be responsible for stress-responsive gene expression with additional regulation of signal transduction (Figure 1.5). The results suggested that there is a combined regulatory system which

controls ABA, drought response and gene activation (Shinozaki & Yamaguchi-Shinozaki, 2007). However, Joo *et al.* (2013) showed that the rice *ASR* gene family includes two genes, *OsASR1* and *OsASR3,* which are induced by drought stress. Results showed that ABA and GA were involved with the regulation of both *OsASR1* and *OsASR3* expression. Furthermore, the overexpression of *OsASR1* or *OsASR3* in transgenic rice enhanced their tolerance to drought stress.

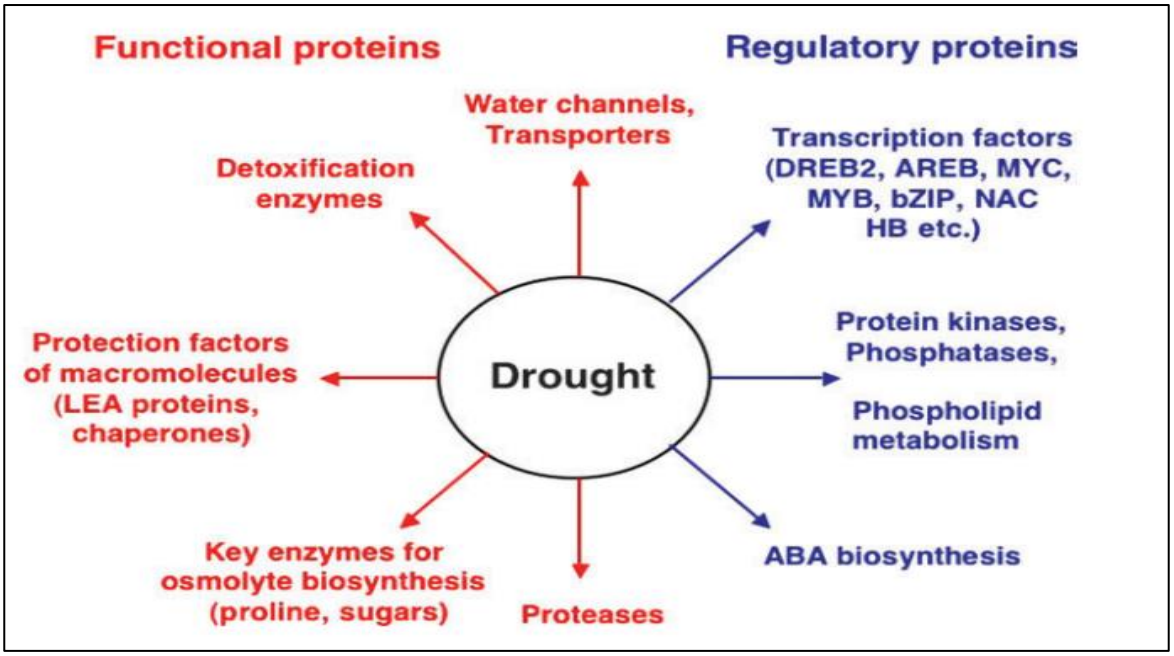


Figure 1.5. Functional proteins and regulatory proteins two groups genes producer. Images from Shinozaki *et al.,* 2007.

In transgenic rice which overexpressed a gene called SNAC1 (STRESS-RESPONSIVE NAC 1) under severe drought, the transgenic lines had 23.0–34.6% increased spikelet fertility compared with wild type which had almost no seed. During a drought period, *SNAC1* gene activate in guard cells to encode a set of TFs such as NAM, ATAF, and CUC (NAC). The overexpression of the *SNAC1* gene is highly linked with the increase of the stomatal closure and ABA sensitivity. This means rice lines with *SNAC1* gene losing water more slowly and this can be vital during drought stress (Shinozaki *et al.*, 2003, Hu *et al.*, 2006, Shinozaki & Yamaguchi-Shinozaki, 2007). Another member of the rice NAC gene family,

OsNAC10, was also shown to be upregulated in response to drought stress (Jeong *et al.*, 2010). Overexpression of *OsNAC10* gene in 14-days-old Nipponbare seedlings resulted in greater tolerance to drought stress (Jeong *et al.*, 2010).

This expression for this gene was induced by the 100 mM ABA treatment, especially in the root. In contrast, non-transgenic rice seedlings expressed visual drought damage such as leaf rolling and concomitant loss of chlorophylls. This implies, rice root response to drought stress through ABA sensing results in activating OsNAC10 gene which improves drought tolerance under field drought conditions (Jeong *et al.*, 2010).

1.10. PGRs and plant adaptations to drought

PGRs can help plants respond to drought (Zhang *et al.*, 2006, Verma *et al.*, 2016). Plant adaptations to drought can be divided into two groups. The first group of adaptations are stress avoidance (dehydration avoidance) mechanisms, which aim to save water inside the plant and balance it with the surrounding environment which means plants are adapting the cellular system to avoid the water deficit. With these mechanisms, the plant can adapt to the short-term stress by restricting shoot growth, accelerating leaf senescence and limiting water loss through evaporation by closing stomata (Blum, 2005, Yue *et al.*, 2006). All these mechanisms aim to reduce water loss from plant tissues and are regulated predominantly by ABA and cytokinins. However, this adaptation is for short term (hours) drought stresses only. The second group of adaptations are for long term (days) drought stress (dehydration tolerance), where the plant will utilise drought stress tolerance mechanisms that are aimed at protecting the most important parts in the plant and adapt to the cellular water deficit. By protecting the cellular system inside the plant and limiting growth, which aims to sustain or conserve plant function, a plant can

fight against severe drought conditions to survive. This mechanism can be the second defence line for the plant after dehydration avoidance was applied (Schulze & Küppers, 1979, Orcutt, 2000, Price *et al.*, 2002, Mäser *et al.*, 2003, Blum, 2005, Nakashima *et al.*, 2009).

Roots are usually the first part of the plant to experience water stress and will signal to the rest of the plant. The sensing action starts with the root cap, where it responds to moisture and gravity signals. These signals are essential for plants to adapt their growth appropriately (Aiken & Smucker, 1996, Mäser *et al.*, 2003, Eapen *et al.*, 2005).

At the genetic level, insights into the mechanisms of this sensing mechanism were obtained through a semi-dominant mutant isolated from Arabidopsis, which had a non-hydrotropic response (nhr1) to water deficit compared with wild type-roots (Eapen *et al.*, 2003). This mutant preferred to extend roots toward low moisture potential. It was suggested that these mutants have altered sensitivity to moisture by reducing ABA and auxin transport processes (Eapen *et al.*, 2003). Consequently, more ABA can be produced and delivered to the affected leaves, where it acts to control the opening and closing of stomata. In addition to regulating stomatal apertures, under severe drought stress, ABA can also reduce wall extensibility of growing cells, and reduce plant growth (Tardieu *et al.*, 2010).

Optimal root systems for capturing water have been proposed for different crops, which focus on deeper root systems to access more water (Lynch *et al.,* 2013; Uga *et al.*, 2013). Lateral root numbers and length are also important, especially further down the soil profile for capturing water at depth (Yu *et al.*, 2015).

However, the response of the root system both spatially and temporally to different severities of drought stress are not fully understood (Bao *et al.*, 2014). One of the

obstacles in sowing rice seeds directly is the difficulty in obtaining uniform emergence of the seedlings.

The early and uniform establishment for seedling could have many advantages, including the ability to establish a longer and larger root system. Such modification can allow them to access water at depth for a longer period of time and increase their tolerance to drought conditions (Ingram & Malamy, 2010, Rich & Watt, 2013).

1.10. Rice production and future challenges

Rice is a staple food for nearly half of the world's population (IRRI, 2010). Annual global rice consumption from 1960 to 2011 has tripled from 150 million tons to approximately 500 million tons (Seck *et al.*, 2012). The United Nations projects that the world's population will reach 9.7 billion by 2050, and 11.2 billion by 2100 up from nearly 7.3 billion in mid-2015 (UN, 2015). Therefore, by 2035 an additional 120 million tons of rice will be required to cover the projected increase in global population. Concurrent with this increase in population there will be an increasing demand for freshwater to cover the need for each individual person (Cosgrove & Rijsberman, 2014). Rice production consumes approximately 40% of the world's irrigation water and 30% of the world's freshwater resources (Qin *et al.*, 2006, Jagadish *et al.*, 2008, Zhang *et al.*, 2008). The increasing pressure of water scarcity has the potential to jeopardise irrigated rice production. Therefore, developing efficient irrigation water system is highly recommended (Greenwood *et al.*, 2010). Increasing rice production globally, will need concerted efforts. This can partly be achieved through varietal development, breeding varieties to cope with climate change, drought and salinity (Seck *et al.*, 2012). Most rice varieties, especially modern varieties, have a potential to produce high yields, but environmental limitations reduce this potential and creating yield gap in the field (Tran, 2004). These environmental limitations reduced rice production in long-term experiments

by a half to a third in some Asian regions, although, best management practices have been intensively applied (Dobermann & Fairhurst, 2000, Uga *et al.*, 2013). Drought stress has already significantly affected rice production in Asia. More than 40 million ha of rainfed lowland and rainfed upland rice are likely to be exposed to drought stress (Wopereis *et al.*, 1996). However, there are several challenges which have inhibited the progress in developing rice genotypes which could be drought tolerant. The two biggest challenges is the lack of information about the root system of rice genotypes and lack of efficient screening techniques (De Dorlodot *et al.*, 2007).

1.11. Rice root system form and function

Previously, scientists and breeders were interested in meeting the demands of food production by modifying the aboveground parts of the plant (Lynch, 2007). Root systems are the main pathway to provide water and nutrients for plants from the soil. Therefore, switching attention from the aboveground to below-ground is the next step for scientists to meet the increasing demand on food (Den Herder *et al.*, 2010, Bishopp & Lynch, 2015). A better understanding of root systems under different environmental stresses will be a crucial factor in improving our understanding of plant adaptation processes (De Smet *et al.*, 2012). Root systems are formed from the interaction between environmental factors and plant genetics. Therefore, analysing and understanding the correlation between seedling root and adult root traits under field conditions is essential in understanding root responses to soil conditions and the G×E interaction (Rich & Watt, 2013). Furthermore, improving resource uptake for plants will be the key to improve or develop plant breeding programmes for future environmental stresses (Tron *et al.*, 2015). This could be achieved through reshaping root systems (Blum, 2009, Den Herder *et al.*, 2010, Kell, 2011, Bishopp & Lynch, 2015). For example, depending on soil water

availability and the genetic potential of the plant (GxE), root systems can have different architectures in the soil (Mai *et al.*, 2014). In soils with high water availability, roots tend to increase their density in the upper layers of the soil profile near the surface. In soils with water deficits, roots tend to descend deeper into the soil depending on variety, root longevity and soil texture (Mai *et al.*, 2014).

Establishing a strong and effective root system during the first stages of plant growth has an enormous influence on the later stages of growth and has been linked with final yield under drought conditions (Hochholdinger & Tuberosa, 2009). Primary root and post-embryonic root development, which is formed after germination from existing roots tissues are the most important stages during root development (Atkinson *et al.*, 2014, Tian *et al.*, 2014). In these growth stages, the root system development is important to ensure good root establishment for later growth stages. Therefore, changes to these initial stages can affect later root system size and architecture. Furthermore, root system architecture, specifically root growth angle (RGA), can be used as a parameter in plant breeding for the evaluation and selection of genotypes. However, other soil restrictions such as salinity and alkaline soils can also negatively restrict root growth (Lynch, 1995, Marschner, 1995, Gregory, 2008).

In a study conducted by Li *et al.* (2017) 529 rice accessions were used to identify the genetic control basis of the root system under normal and drought stress conditions, whole genome association was used (WGAS) in this study. At the booting stage drought stress was applied until the plant leaves fully rolled, then recovered. The results identified 413 associations, 143 of them were suggested to be linked with 21 root traits, such as maximum root length, root volume, and root dry weight. Alongside of 11 active root-related genes such as, *DRO1*, *WOX11*, and *OsPID* were sharing the association same location. *Nal1* and *OsJAZ1* were the two

suggested genes to control of root traits based on this association. As well, the mean of maximum root length, under the drought condition was 49.2 cm compared to 48.8 under normal conditions.

Plant root systems have been described and explained in many different ways, which can be confusing (Gregory, 2008). Since there are different descriptions for root systems, for the purposes of this thesis, the seedling rice root system will use the following terminology (Figure 1.6). Root systems for monocotyledons, contain a primary root (PR), lateral roots (LR), and in some species aerial roots (AR). The PR is defined as the first root emerging from the seed. Lateral roots are defined as those emerging from the PR. Measuring LR numbers is challenging, especially during the later stages of plant development (De Smet *et al.*, 2012). The AR are defined as roots emerging above the ground parts of a plant. These are sometimes also referred to adventitious or crown roots in the literature.

Most previous studies have focused on Arabidopsis as a model for dicotyledonous plant to characterize root traits under different environments. However, rice is considered as model for monocotyledons plants with small genome size and a known genome sequence. Therefore, investigating root systems in rice can provide better understanding for the complexity of root systems (Itoh *et al.*, 2005, Den Herder *et al.*, 2010, De Smet *et al.*, 2012, Lavenus *et al.*, 2013).

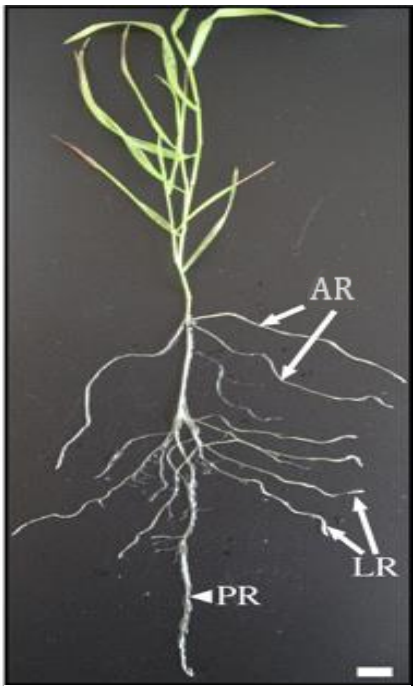


Figure 1.6. Monocotyledon sample root system architecture after 30-day *Brachypodium* plant, PR, primary root; LR, lateral root; AR, aerial root. (Pacheco-Villalobos & Hardtke, 2012).

1.12. Methodologies for root studies in rice

Root system characteristics and their quantification are still a key factor for understanding plant performance, as there is still a need to understand the factors that influence or possibly enhance them (Judd *et al.*, 2015). Studying root systems is still a big challenge, therefore there is still a need to fill a knowledge gap about the mechanism of drought-resistant, drought avoidance and root traits (Li *et al.*, 2017). Although many studies have been carried out on plants root systems, none have linked the effect of PGRs on the rice root system with drought and variation in these responses between genotypes. Therefore, identifying an appropriate method to study rice root systems is important to evaluate the performance of different genotypes under drought conditions.

There are different methods to study rice root systems. Some of them are suitable for glasshouse, others are appropriate for field studies and some can be used in both (IRRI, 2010). Root sampling and quantification can be achieved under controlled environment conditions by using a root box–pin-board method (Figure 1.7, (Kano-Nakata *et al.*, 2011), filled glass rhizotrons for visualizing roots (Figure 1.8), PVC tubes (Figure 1.9), quantitative measurement of root growth angle by using the basket (Figure 1.10), assessing root growth and water extraction by maintaining water at different depths (Figure 1.11) and assessing root penetration ability and resource capture from deeper soil layers (Figure 1.12). For field studies, root sampling can be achieved using soil cores and monoliths, the raised-bed system and deep root restriction system, a unique method of screening deep-rooted genotypes in the field (Shashidhar, 2012, Henry, 2013, Judd *et al.*, 2015). The most modern methods of measuring the root system are the use of computer tomography (CT) scanning techniques (3D CT X-ray imaging) in combination with a robot, which has been specifically designed to meet the special needs of root imaging (Figure

1.13, (Metzner *et al.*, 2015). Using software to analyse images of root systems is considered an effective and cheaper option to analyse various root growth characteristics, from root length to number of lateral roots to total root length (Clark *et al.*, 2011, De Smet *et al.*, 2012). Each of the above methods has advantages and disadvantages, which have been previously explained (Henry & Hardy, 2012). For simulating rice field conditions with the ability to measure root length and root dry weight under normal and drought conditions, the PVC method was used in this study. Using soil moisture sensors would add another factor to use PVC method over other methods. Root sampling by using root box–pinboard and soil-filled glass rhizotrons methods was in consideration alongside other methods to be used in this project. Nevertheless, because of restrictions by growth room size, experimental design and practical requirements PVC method was more appropriate to be used.

Figure 1.7. Pinboard box method for assessing rice root systems. Using polyethylene sheets for holding roots after the soil has been washed out. Images from Kano-Nakata *et al.,* 2011.

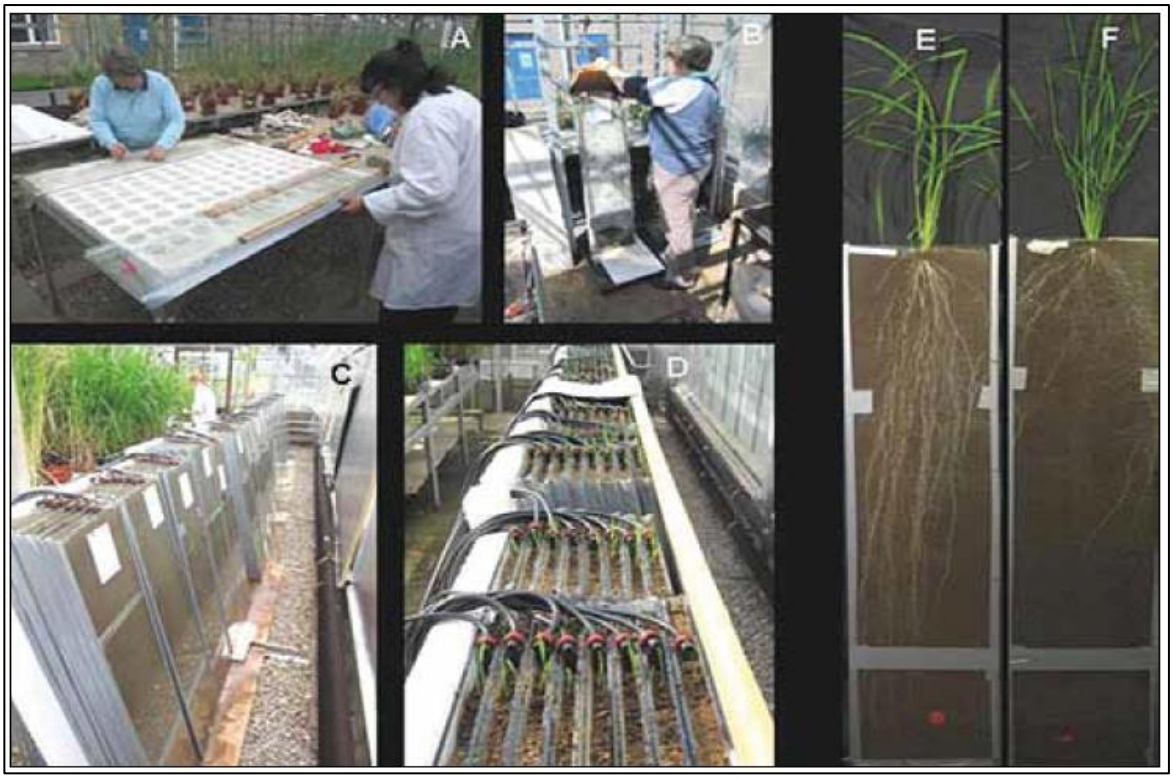


Figure 1.8. Rhizotron method for assessing rice root systems. Using rhizotron box which be stacked at 15º. Images from PRICE *et al.,* 2012.

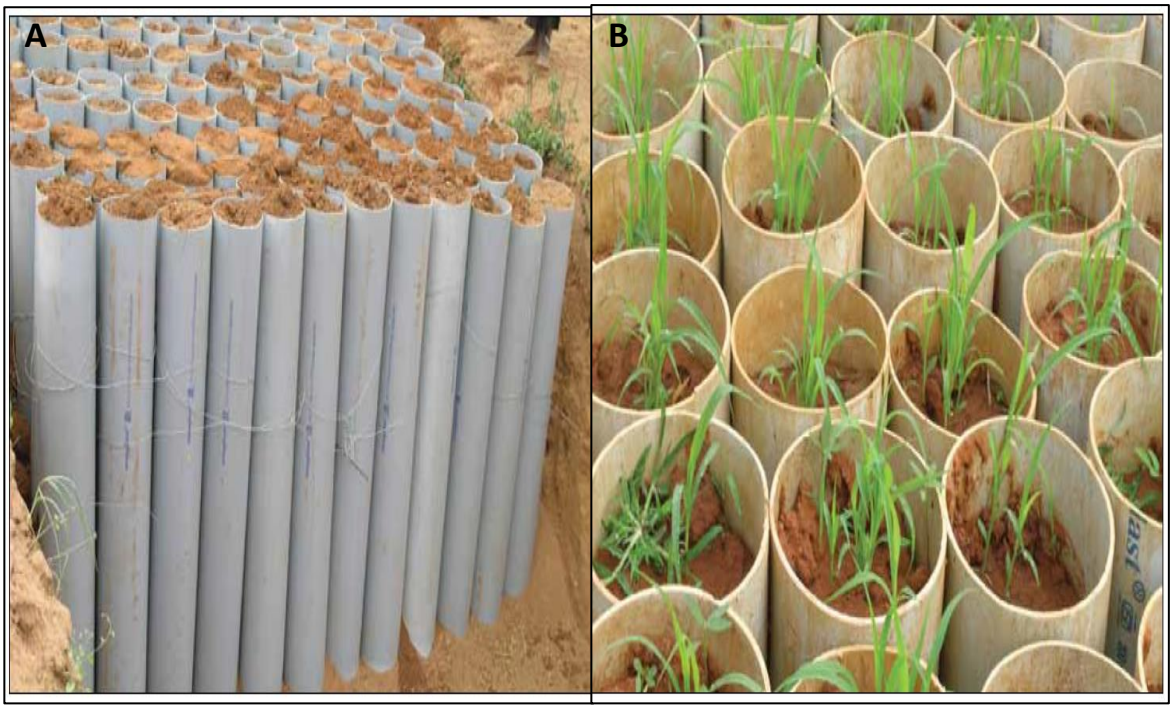


Figure 1.9. Using PVC tubes for assessing rice root systems. (A) PVC filled with soil, (B) Growing seedlings. Images from Shashidhar *et al.,* 2012.

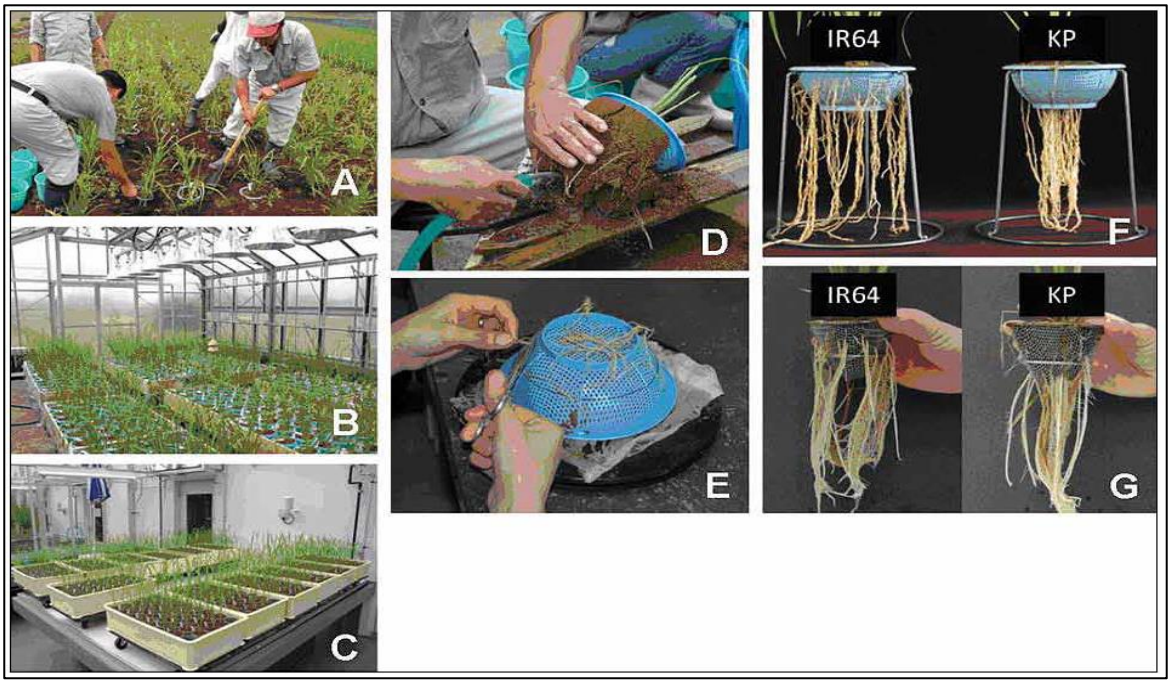


Figure 1.10. Using plastic basket for measuring root growth. (A) plastic baskets after been removed out from field. (B, C) Hydroponic culture system for growing rice. (D) Washing the soil out. (E) Roots counting. (F, G) Variation between the two genotypes IR64 and Kinandang Patong in root growth angle. Images from UGA, Y. 2012.

Figure 1.11. Using PVC cylinders with side holes for sensors insertion to monitor soil moisture. Images from WADE, *et al.,* 2012.

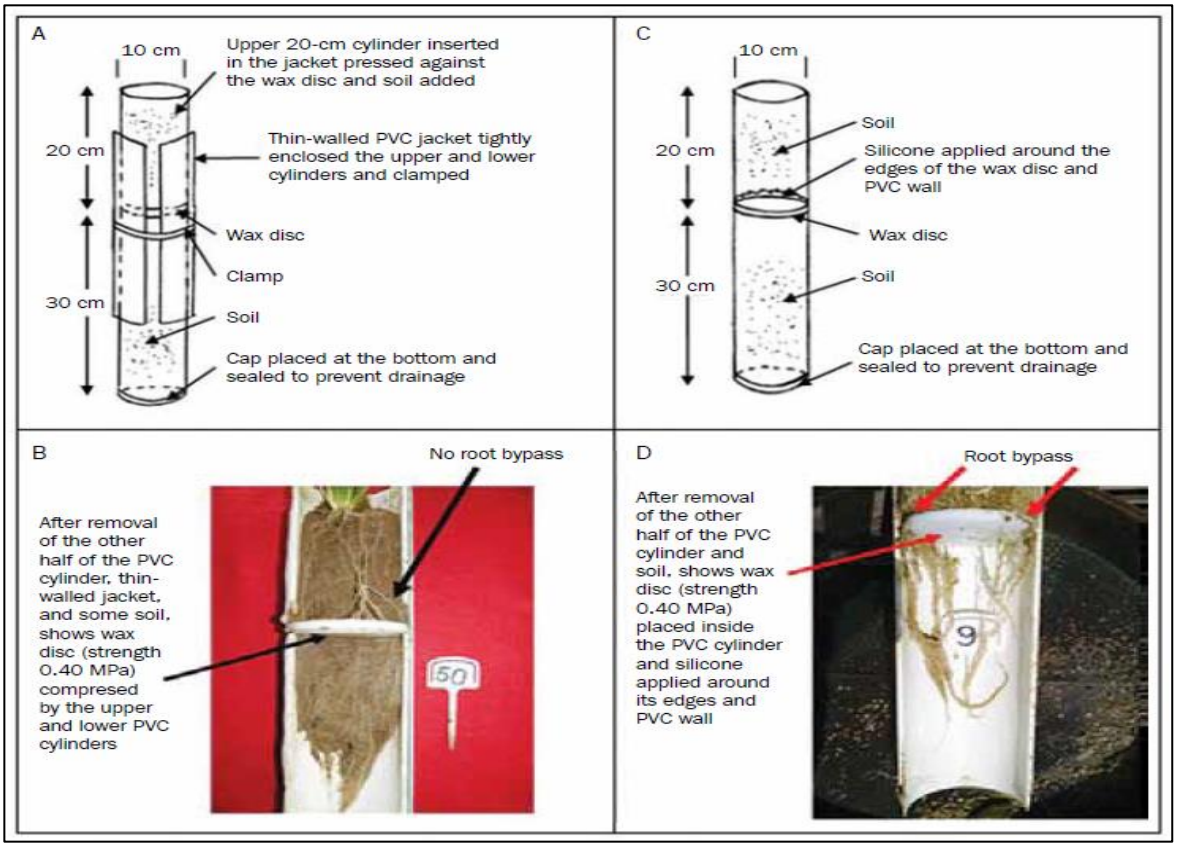


Figure 1.12. Assessing root penetration ability method. Images from WADE, *et al.,* 2012.

Figure 1.13. 3D X-ray robot imaging method. The University of Nottingham https://www.nottingham.ac.uk/microct/facilities/vtomexl.aspx.

1.13. Project overview

Tolerance to drought is a complex process and demands a combination of different strategies to overcome (Fleury *et al.*, 2010). One of the hypotheses is that, increasing the root length of plants can allow them to access water at depth for a longer period of time and increase their dehydration avoidance conditions (Rich & Watt, 2013, Vadez, 2014). Consequently, water use by plants will be maximised and reduced the water percolation to aquifers. Superior understanding of root traits can fill the knowledge gaps of root responses to environmental factors and provide plant breeders with information to develop plants better adapted to drought (Rao *et al.*, 2016). Root systems can be improved through different techniques such as breeding, transgenic approaches and chemical enhancement of the root system by using PGRs (Ferguson & Lessenger, 2006). However, many crops cannot maximise yield without sufficient amounts of water. Therefore, the future challenge is to increase, or in the worst case maintain, crop production while trying to save water resources under increasing population growth. (Ehlers & Goss, 2016).

Aim:

The aim of this research is to investigate to what extent plant growth regulators could enhance drought tolerance in rice through establishing a more effective root system during the initial stages of plant growth.

Objectives:

- To test the effect of different PGRs on rice seedling growth and root traits.
- To determine the variability in responses to PGRs across a diverse set of rice germplasm.
- To investigate the impact of PGRs on drought stress for one variety in soil from seedling to maturity.
- To assess the effect of seed treatment with PGRs on gene expression and the expression of genes related to drought stress.

2. Material and methods

2.1. Plant material

Twenty varieties of rice (*Oryza sativa* L.) were obtained from the International Rice Research Institute (IRRI, Los Baños Research Centre, Los Baños, Laguna Philippines) in 2014. The varieties were selected according to country of origin, days to maturity, variety group, drought-vigour and drought recovery scores (Table 2.1).

Two preliminary experiments were conducted to determine the optimal conditions for sterilising IR64 rice seeds without negatively affecting subsequent germination or seedling growth and the optimal time to germinate the IR64 rice seeds to a size that can be measured accurately. Seeds were subsequently sterilised for 10 mins with 15% (v/v) domestic bleach which contains 4.5/100g of NaOCl followed by three washes with autoclaved distilled water (Chun *et al.*, 1997, Anuradha & Rao, 2003). Three time points, 5, 7 and 9 days, were chosen to test the best growing time.

2.2. Plant growth regulators (PGRs)

Six PGRs were chosen to represent three groups of PGRs, indole-3-acetic acid (IAA) and 1-Naphthaleneacetic acid (NAA) to represent the auxin group; 6-benzylaminopurine (BA), kinetin and zeatin to represent the cytokinin group; and gibberellic acid potassium salt (GA3) to represent the gibberellin group. Gibberellin, Kinetin, IAA and NAA were obtained from Sigma-Aldrich (Sigma-Aldrich, Dorset, UK). Zeatin was obtained from MP Biomedicals (MP Biomedicals, UK).

Table 2.1. Information about the rice genotypes obtained from International Rice Research Institute (IRRI).

NO.	Country of origin	Accession number	Scientific name	Acquisition date	Also known as	Days to Maturity	Variety Group	Drought - Vigor	Drought recovery	Holding institute	Reference
1	Taiwan	IRGC 3541	*Oryza sativa*	19770115	FEE LA FON	102	JAPONICA	NORMAL	SLOW	PHL015	IRRI website
2	Japan	IRGC 5320	*Oryza sativa*	19790620	YAKUMO	84	JAPONICA	NORMAL	INTERMEDIATE	PHL020	IRRI website
3	Iraq	IRGC 9503	*Oryza sativa*	19631206	NAYIMA	138	INDICA	WEAK	INTERMEDIATE	PHL003	IRRI website
4	Iraq	IRGC 9504	*Oryza sativa*	19631206	GHRAIBA	128	INDICA	WEAK	INTERMEDIATE	PHL004	IRRI website
5	Iraq	IRGC 9505	*Oryza sativa*	19791204	AMBAR	112	INDICA	WEAK	INTERMEDIATE	PHL005	IRRI website
6	Iraq	IRGC 9506	*Oryza sativa*	19791204	BAZIAN	108	INDICA	WEAK	INTERMEDIATE	PHL006	IRRI website
7	Taiwan	IRGC 10309	*Oryza sativa*	19790919	KAOHSIUNG 136	90	JAPONICA	WEAK	INTERMEDIATE	PHL014	(Lin, 1993)
8	India	IRGC 19379	*Oryza sativa*	19730628	N 22	91	INDICA		INTERMEDIATE	PHL010	(Selote & Khanna-Chopra, 2004, Prasad *et al.*, 2006b, Jagadish *et al.*, 2008, Jagadish *et al.*, 2010)
9	Philippines	IRGC 23364	*Oryza sativa*	19730831	KINANDANG PATONG	125	INDICA		FAST	PHL008	(Puckridge & O'Toole, 1980, Uga *et al.*, 2013)
10	Thailand	IRGC 23717	*Oryza sativa*	19731001	DAW PAO	90		VIGOROUS	SLOW	PHL018	IRRI website
11	Iraq	IRGC 26897	*Oryza sativa*	19750208	CHOUL	98	INDICA	NORMAL		PHL001	IRRI website
12	Iraq	IRGC 26898	*Oryza sativa*	19750208	GHRAIBA 52	98	INDICA	NORMAL		PHL002	IRRI website
13	Bangladesh	IRGC 29087	*Oryza sativa*	19731015	AUS 299	98	INDICA	EXTRA VIGOROUS	INTERMEDIATE	PHL013	(Swamy & Kumar, 2012)
14	Japan	IRGC 30322	*Oryza sativa*	19750219	KOKO 13	85	JAPONICA	WEAK	VERY FAST	PHL019	IRRI website
15	Pakistan	IRGC 76317	*Oryza sativa*	19881110	BASMATI 385	119	INDICA			PHL012	(Akram *et al.*, 2013)
16	China	IRGC 77442	*Oryza sativa*	19890427	HE JIANG 16	77	JAPONICA			PHL016	IRRI website
17	Korea	IRGC 77639	*Oryza sativa*	19890102	CHALBYEO	80	JAPONICA			PHL017	(Yan *et al.*, 2011)
18	Bangladesh	IRGC 87165	*Oryza sativa*	19961111	AUS BAK TULSI	113	INDICA			PHL011	(Swamy & Kumar, 2012)
19	Philippines	IRGC 115128	*Oryza sativa*	20041210	NSICRC9					PHL009	(Suralta & Yamauchi, 2008)
20	Philippines	IRGC 116793	*Oryza sativa*	20050516	IR64					PHL007	(Jury & Vaux, 2005, Liu *et al.*, 2006, Jagadish *et al.*, 2008, Jagadish *et al.*, 2010)

2.3. Effect of PGRs on IR64 rice genotype seedling growth

Seedling growth assays were conducted to investigate the effect of soaking seeds for 24 hours in different PGRs. Three biological replicates were performed, separated by time, for each PGR and concentration. For each PGR and concentration within a biological replicate, eight seeds of IR64 were treated with either 0, 100, 250, 500 and 1000 µM of IAA, NAA, BA, GA3 and Kinetin for 24 hours prior to germination. PGR treatments were prepared fresh from stock solutions for each biological replicate. Stock solutions were prepared fresh for each experiment, IAA, NAA, kinetin, GA3 and zeatin were prepared by adding the PGRs to the appropriate solvents (Table 2.2). PGRs and their solvents were vortexed for 5 minutes, to dissolve the PGRs. The stock was then diluted to obtain concentrations of 100, 250, 500 and 1000 µM for each PGR (Table 2.2).

Sterilised seeds were soaked in separate pre-prepared 5 mL Eppendorf tubes filled with 4.5 mL of specific PGR concentration and rotated end-over-end at 35 rpm for 24 hours. The seeds were subsequently rinsed with autoclaved water (ADW). Seeds were placed on a white lab tissue for 5 minutes after they were removed from PGRs solutions, prior to germinating them on moist paper towels (Figure 2.1). The paper towel roll system was used in this study with some adjustment to suit image scanning (Zhu *et al.*, 2005, Bai *et al.*, 2013). The paper towel system was used because it allowed the root and shoot to grow in either direction without restrictions. Seeds were germinated on moist HOSTESS 230 mm x 310 mm paper towels and left to grow for 7 days inside the incubator with conditions 16h/8h light/dark and 34/11°C day/night (Ueno & Miyoshi, 2005). Rolled paper towels were kept in plastic bags to maintain the moisture before being placed in incubator. After 7 days, shoot and root lengths, aerial root numbers, lateral root lengths and numbers and total seedling weight were recorded.

For imaging purposes and to simplify the measurements later (see section 2.6), germinated seeds were removed from the paper towels and placed on black cardboard with scale marker on the side (Figure 2.2). A randomised complete block design (RCBD) was used in all seedling experiments to randomise the location of treatments within the incubator, where blocks were placed randomly at the front and back, left and right corners on the incubator shelf.

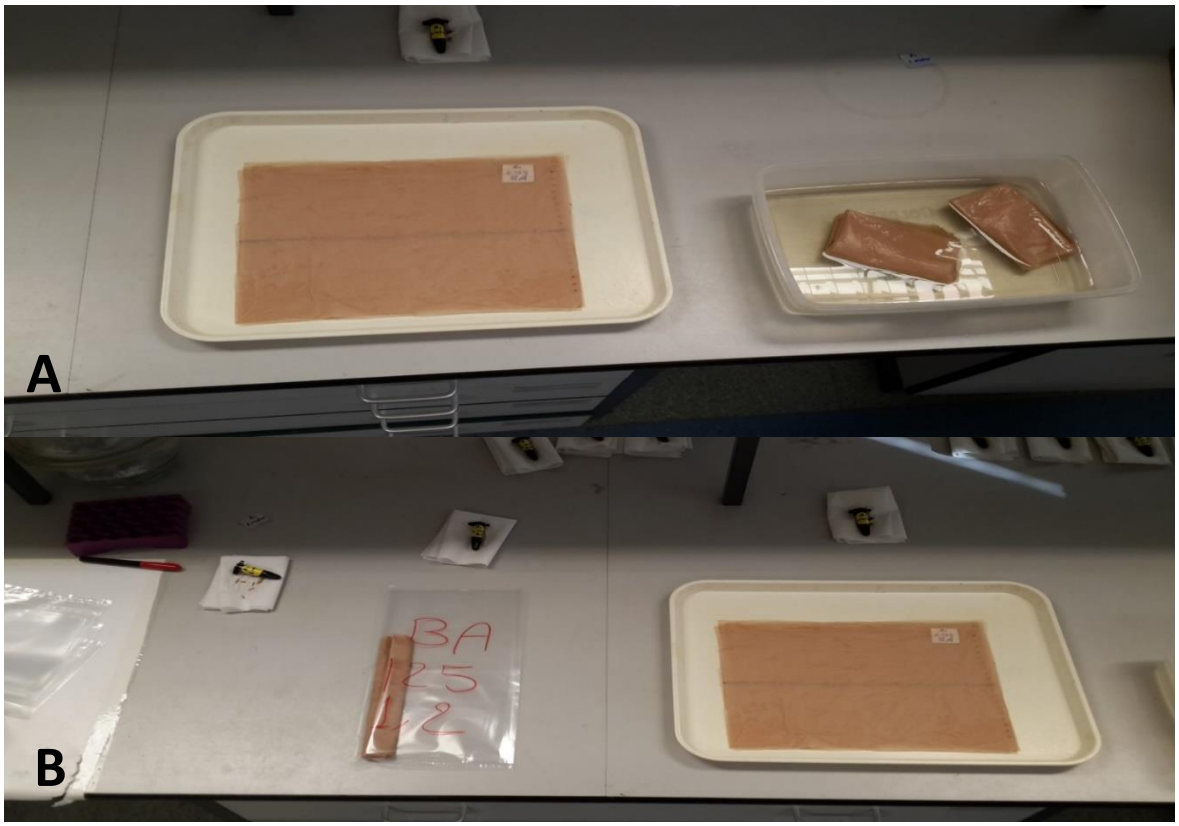


Figure 2.1. Seedling screen experimental set up. HOSTESS 230mm x 310mm paper towels were soaked in autoclaved distilled water and squeezed with medium hand pressure and opened to place the treated seeds (A). Rolled paper towels placed inside a plastic bag before putting in the incubator after located vertically in a metal basket mesh, each roll contains twelve seeds (B).

Table 2.2. Stock preparation, solution and dilution for PGRs.

PGR Group	PGR Amount	Molecular Weight g/mol	Solvent	Diluent	Concentration µM	PGR	Stock Concentration	Stock Volume	Grams of Hormone
Auxin	**IAA**	197.17	Water	Water	125	**IAA**	100mM	25mL	0.492
	2g	197.17	Water	Water	250	2g			
		197.17	Water	Water	500				
		197.17	Water	Water	1000				
	NAA	186.21	1N NaOH	Water	125	**NAA**	100mM	25mL	0.466
	25g	186.21	1N NaOH	Water	250	25g			
		186.21	1N NaOH	Water	500				
		186.21	1N NaOH	Water	1000				
Cytokinin	**Kinetin**	215.21	1N NaOH	Water	125	**kinetin**	100mM	25mL	0.538
	1g	215.21	1N NaOH	Water	250	1g			
		215.21	1N NaOH	Water	500				
		215.21	1N NaOH	Water	1000				
	BA	225.25	1N NaOH	Water	125	**BA**	100mM	25mL	0.563
	1g	225.25	1N NaOH	Water	250	1g			
		225.25	1N NaOH	Water	500				
		225.25	1N NaOH	Water	1000				
	Zeatin	219.2	1N NaOH	Water	125	**zeatin**	2mM	22.8102 mL	0.01
	10mg	219.2	1N NaOH	Water	250	10mg			
		219.2	1N NaOH	Water	500				
		219.2	1N NaOH	Water	1000				
Gibberellin	**GA3**	384.46	Water	Water	125	**GA3**	100mM	25mL	0.961
	1g	384.46	Water	Water	250	1g			
		384.46	Water	Water	500				
		384.46	Water	Water	1000				
		384.46	Water	Water	3000				

2.4. Variation in rice seedling growth between twenty genotypes

Three biological replicates were performed and separated by time. For each biological replicate, 12 seeds of each genotype were soaked for 24 hours in ADW prior to germination. Seeds were soaked in separate pre-prepared 5 mL Eppendorf tubes filled with 4.5 mL of ADW and rotated end-over-end at 35 rpm for 24 hours. Seeds were subsequently sown on paper towels and grown as described previously (see section 2.3).

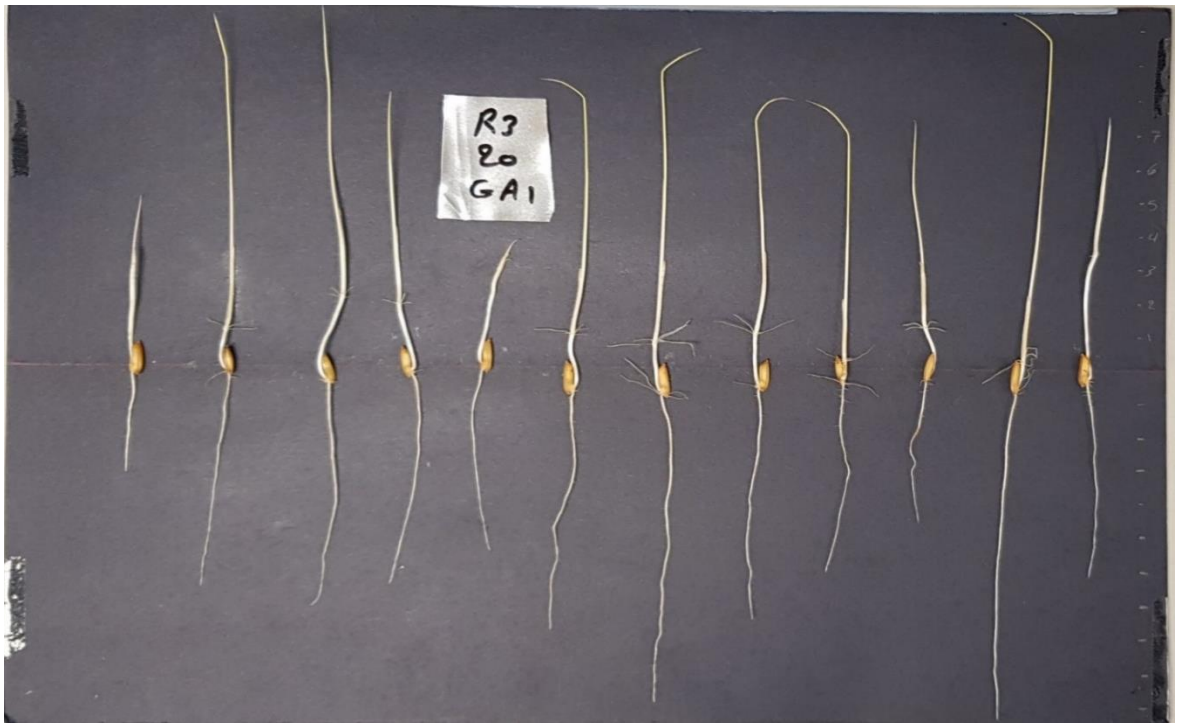
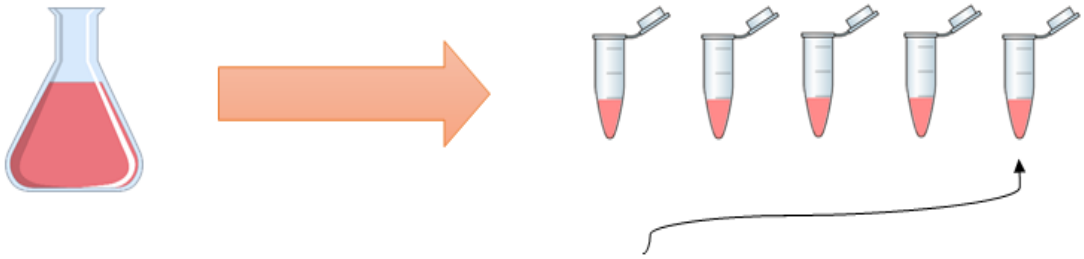


Figure 2.2. Seedlings were placed on a black cardboard with drawn scale on the side. This scale was used to convert pixels distance to centimetre.

2.5. Variation in responses to PGRs between rice genotypes:

Three biological replicates were performed and separated by time. For each PGR and concentration within a biological replicate, 12 seeds of each genotype were treated with either 1000 µM GA3, 3000 µM GA3, 250 µM NAA, 125 µM BA or ADW as a control for 24 hours prior to sowing (Figure 2.3). PGR treatments were prepared fresh from stock solutions for each replicate. Seeds were soaked in separate pre-prepared 5 mL Eppendorf tubes filled with 4.5 mL of specific PGR concentration and rotated end-over-end at 35 rpm for 24 hours. The seeds were subsequently rinsed with ADW and sown on paper towels and grown as described previously (see section 2.3).

Plant hormones	Stock Concentration	Stock Volume	Grams of Hormone	Needed concentration	From stock concentration	ul stock	mL ADW	Total Volume	Final Volume
NAA	100 mM	25mL	0.466	**250um**	0.02	20	9.98	10	6
BA	100 mM	25mL	0.563	**125um**	0.01	10	9.99	10	6
GA3	100mM	25mL	0.961	**3000um**	0.3	300	9.7	10	7



Plant hormones	Needed concentration	mLs of last GA3 dilution	mL ADW	Total Volume	Final Volume
GA3	**1000um**	3	6	9	5

Figure 2.3. Preparation of different concentrations of different plant growth regulators (PGRs), stock and dilution process to the wanted concentrations, ADW, autoclaved distilled water.

2.6. Analysis of rice root and shoot traits through image analysis

Images of seedlings on paper towels were taken using Sony Cyber-shot DSC-HX20V digital camera. Images were stored as JPG images. Three software packages were used to analyse the image:

1- ImageJ 1.50b: Wayne Rasband, National Institutes of Health, USAJava 1.6.0_24 (64-bit) http://imagej.nih.gov/ij.

2- SmartRoot, version 4.21, 2014-04-11. Software created by Xavier Draye, and Guillaume Lobet, Université Catholique de Louvain, https://smartroot.github.io/, (Lobet *et al.*, 2011).

3- RootReader2D v4.3.1 software: Randy Clark/ USDA/ARS-Cornell University http://www.plantmineralnutrition.net/ (Clark *et al.*, 2013).

ImageJ software was used to measure image pixels and convert the pixels to centimetre scale. This was achieved by using a straight segmented or freehand line, or arrows tools to draw a straight line between any two numbers on the side scale, preferably, the two numbers can be the same for all treated photos. Then, from analyse icon and set scale, the distance in pixels was recorded and scaled to cm. Smart-Root was used to convert images to greyscale and saved under the "tif" format to be used in RootReader-2D. Finally, root and shoot traits were subsequently quantified using RootReader-2D (Figure 2.4).

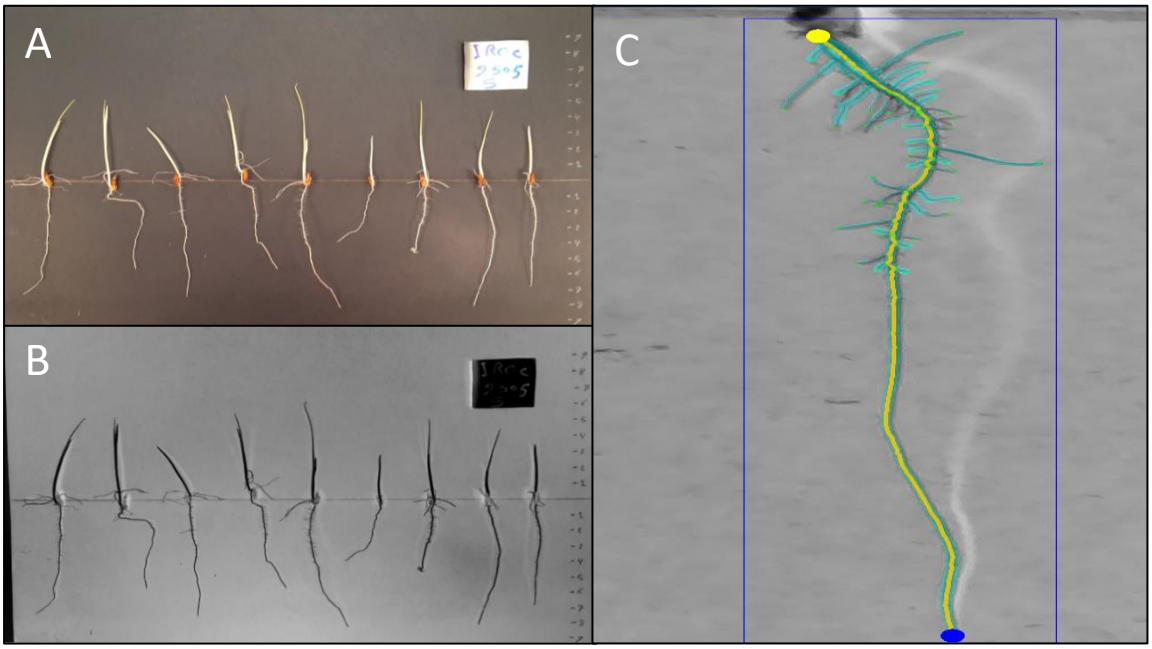


Figure 2.4. Example image of seedlings after 7 d growth (A), which was converted to greyscale using ImageJ/SmartRoot (B) and analysed using RootReader-2D for root traits (C).

2.6.1 Programme setting up

Within RootReader-2D the following options were selected: double adaptive thresholding, use dust removal filter (runs after thresholding) and use filling filter (runs after thresholding and/or dust removal). For root selection the following options were activated: allow any skeleton point to be selected as root endpoint, select roots that share a common endpoint and automatic prediction of furthest endpoint. A selection of 25 pixels were chosen for the dust removal filter and the scale was set.

2.6.2 Programme measurements

After the image was opened in RootReader-2D the twelve seedlings were measured separately for root and shoot length. Regions containing individual roots and shoots were selected and the threshold points option was used to delete all un-wanted areas (Figure 2.5). The skeletonize function was then used to convert plant structures in the highlighted regions. Build segments was then used to add green points on each lateral root. Finally, the measure function was used to extract the measurement and save it in the measuring log (Figure 2.5). To validate this approach, a number of random paper towels were picked to measure the root traits manually. The manually measured traits were compared with the programme measurement to evaluate the programme measurements accuracy. All primary root length measurements were almost the same for the programme and manual results. Minor differences were observed with the lateral root count which was ±5 per replicate. This resulted in the measurements of lateral root length and total root length varying by ± 1% between manual and RootReader-2D measurements. However, manual measurement of root traits for all experiments would not be practical within the expected time for each experiment. Therefore, using these programmes were essential.

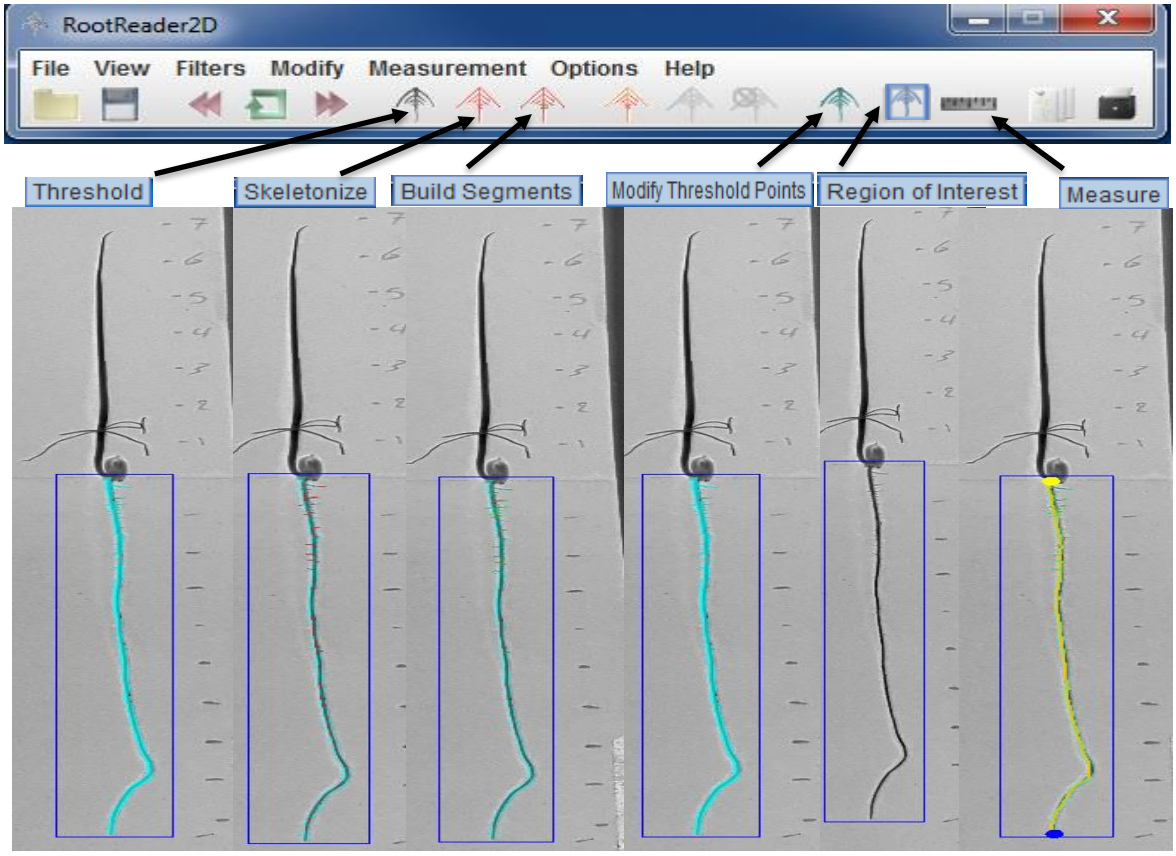


Figure 2.5. RootReader-2D image processing of seedlings root traits and measurements after 7 d growth.

2.6.3 Data analysis

All experimental data obtained from analysis of images were analysed by using GenStat version 16.1.0.10916 (VSN International Ltd. UK). Global analysis of all data was conducted using correlation analysis between traits and via Principal Component Analysis (PCA). PCA analysis was based on the Sum of Square and Products analysis. To investigate differences between individual PGRs, concentrations and genotypes, a Residual Maximum Likelihood (REML) analysis was conducted with PGR*Concentration*Variety as the fixed model and Replicate/Block as the random model.

Chapter 3. Variation in the seedling growth responses of different rice genotypes to seed treatments with PGRs

3.1 Introduction

The first stage in seed germination begins with water uptake (imbibition) (Weitbrecht *et al.*, 2011). This stage can be divided into three different phases. Phase I, starts when water is transferred from outside of the seed to the inside. This is because mature seeds have very low water content. Phase II, seeds at this phase can be fully hydrated. More water uptake can occur after the embryo activates physiological processes (Phase III). However, water influx can be affected by seed coat or seed structure. Water influx also causes many changes within the seed, such as ion infiltration and proteins released, such as lectins and proteinase inhibitors, which may protect against bacteria or insect invasion. At the end of the first stage, physiological processes such as, respiration, protein synthesis and solutes transport are started so new growth can begin (Bewley, 2001, Farooq *et al.*, 2009b).

The second stage of seed germination begins when the embryo resumes metabolic activity. Respiration is the first process in the second stage. Initially seeds can start with anaerobic respiration moving to aerobic respiration when oxygen becomes available. However, some seeds are adapted to use dissolved oxygen when they germinate, such as rice seeds. Enzymes and the Krebs' cycle is activated at this stage to produce adenosine tri-phosphate (ATP) (Herman *et al.*, 1981, Ehrenshaft & Brambl, 1990, Botha *et al.*, 1992, Takahashi *et al.*, 2011). The carbohydrates core components are polysaccharides such as cellulose and starch. During seed germination, complex forms of polysaccharide are hydrolysed to simple hexoses, which are essential for protein, lipid, and carbohydrate synthesis.

During seed germination GA works to activate α-amylase to breakdown the stored starch in the endosperm (Wool & Sun, 2011).

In this second stage, germinated seeds are mainly using endosperm storage as a main provider for carbohydrates, oils and proteins until the seedling becomes photosynthetically active. Hydrolysis of starch by amylolysis enzymes is synthesised into amylose and amylopectin. These two types of starch are hydrolysed by a-amylase and b-amylase to produce glucose and maltose. Then, sucrose, hydrolysed by sucrose synthesizing enzymes, is transfer from endosperm to embryo to support growing regions. Protein synthesis is also starting, using mRNA from activated genes. All mRNAs produced at this stage are mainly used to encode essential proteins to support growth (Bewley & Marcus, 1990). Within this stage, PGRs are also activated, with GA released from scutellum and transferred to aleurone layer to activate several hydrolytic enzymes (Figure 3.1).

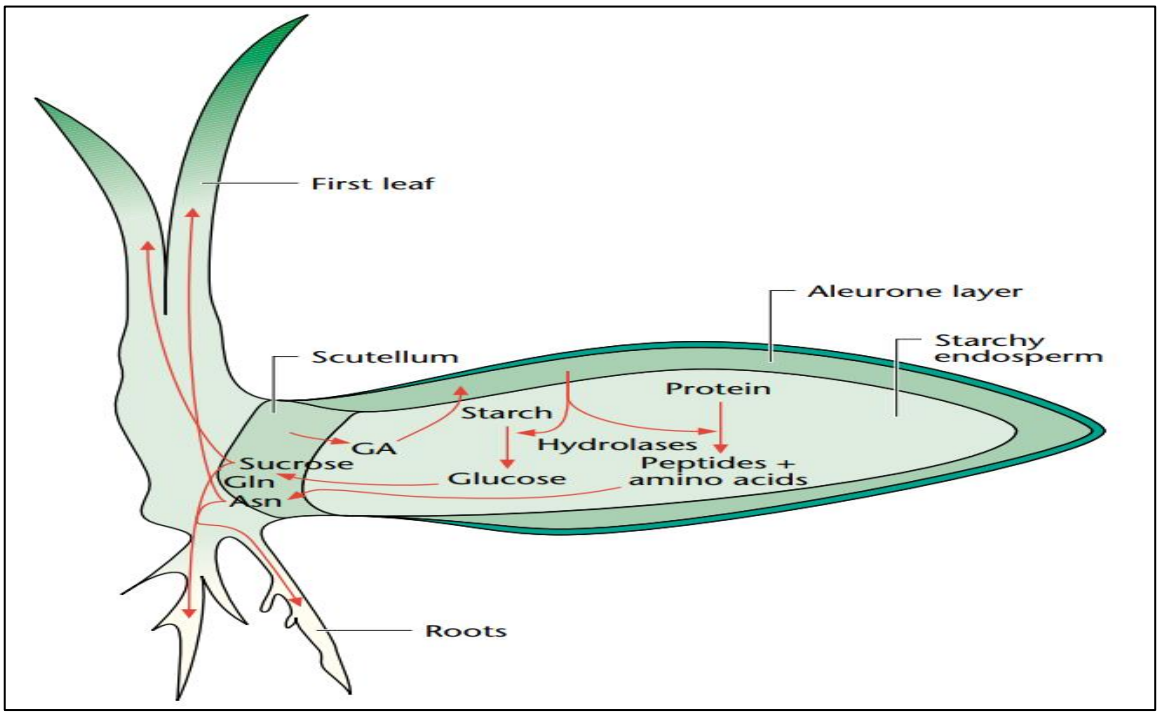


Figure 3.1. Mobilization process during germination and seedling stage for Barley. Diagram shows GA3 role during germination stage as it travels from scutellum to hit Aleurone layer to release hydrolytic enzymes. Image from Bewley, 2001.

The final stage of germination process ends with primary root and coleoptile appearance. Cell division and cell elongation then follow the germination stage (Takahashi *et al.*, 2011). Seed germination and seed development, seedling growth, root proliferation, determination of leaf size and shape, flower induction and development, pollination and fruit expansion can be regulated by gibberellins (GA) (Sun, 2010). However, GA is likely to interact with other PGRs such as ABA (Vishal & Kumar, 2018). Maintaining the balance between GA and ABA is critical for enabling plants to respond to different abiotic stress as well as different plant growth stages (Vishal & Kumar, 2018). Seed germination could be the key starting point for studying PGRs signals (Rajjou *et al.*, 2012, Han & Yang, 2015). As seed germination is a physiological and transition process from dormancy to germination alongside activating PGRs (Liu *et al.*, 2013). Furthermore, using PGRs can improve both rice seedling emergence and crop performance under biotic and abiotic stress (Bari & Jones, 2009, Zhang *et al.*, 2009, Fahad *et al.*, 2016). Soaking rice seeds in PGRs prior to direct seeding can maintain early establishment of uniform seedling and enhance competition with weeds (Lamichhanea *et al.*, 2019). The early treatment with PGRs through vegetative growth results in establishing a vigorous root and shoot system. This has significant advantages over weed competition when growth factors such as light, water and nutrients, become restricted (Lamichhanea *et al.*, 2019). Hence, Kaur *et al.* (2015) demonstrated that soaking rice seeds in gibberellic acid (GA3) at 100 ppm improved germination, root length and seedling establishment. This increase in root length after GA3 application results from activation of hydrolytic enzymes. This may result in dispensed with labour cost for seedling transplanting and save water (Choudhary & Suri, 2014, Kaur *et al.*, 2015). As most lowland cultivated rice using seedbed preparation method, when pre-germinated rice seedlings are transferred to the paddy field condition and this

method requires labour and time (Farooq *et al.*, 2011b). Kim *et al.* (2006) showed that, exogenous application of indole-3-acetic acid (IAA) or gibberellin (GA3) during seed germination had the ability to mobilize the endogenous IAA and GA3 plant hormones and starch under salt stress. This study demonstrates, applying 10 µM GA3 or 20 µM IAA significantly increased the α-amylase activity under NaCl stress for rice seeds compared with control. This means, more starch can be hydrolysed and transferred to the embryo. Furthermore, root length was increased when seeds were soaked in GA3 or IAA compared to the NaCl treatment, however the GA3 treatment increased seedling root length to a similar length as non-stressed control seedlings (Kim *et al.*, 2006). However, there is still significant knowledge gap about how different plant genotypes respond to different seed treatments (seed priming). Therefore, developing precise seed priming techniques still requires testing with different concentrations of PGRs for different durations (Farooq *et al.*, 2009b).

3.1.1 Aim and hypotheses

The aim of this chapter is to highlight the variability between different rice genotypes in responses to PGRs and the effects of soaking seeds in PGRs on rice seedling growth traits.

Hypothesis 1. Soaking seeds in auxins will increase root growth.

Hypothesis 2. Soaking seeds in gibberellins will increase root growth.

Hypothesis 3. Soaking seeds in cytokinins will decrease root growth.

Hypothesis 4. Seedling root and shoot traits will vary significantly between rice genotypes.

Hypothesis 5. Seedling responses to soaking seeds in PGRs will vary significantly between rice genotypes.

3.2 Material and methods

The plants material and methodologies used in this chapter were described in Chapter 2. Briefly, For the twenty genotypes experiment, only three PGRS were chosen (NAA, GA3 and BA) for more investigation, these PGRS represent the main groups which are Auxin, Gibberellin and Cytokinin. Seeds were sterilised for 10 mins with 15% (v/v) domestic bleach which contains 4.5/100g of NaOCl followed by autoclaved distilled water washes three times. The sterilised seeds were then soaked in pre-prepared 5 mL Eppendorf tubes filled with 4.5 mL of specific PGR concentration and rotated end-over-end at 35 rpm for 24 hours. Paper towel roll system was used for germination and seedling assays. Germinated seeds traits were measured by using three software analyses which were ImageJ 1.50b, SmartRoot, version 4.21 and RootReader 2D v4.3.1.

Data analysis

Global analysis of all data was conducted using correlation analysis between traits and via Principal Component Analysis (PCA). PCA analysis was based on the Sum of Square and Products analysis.

3.3 . Results

3.3. Optimisation of seedling growth duration

To test the optimal time for seedlings to grow in the filter paper system and allow root and shoot traits to be measured, IR64 rice seeds were grown for three different periods, 5, 7 and 9 d. (Figure 3.2). Although the seedling root lengths at the 9 d time point were significantly ($P<0.001$) higher than the 7 d time point the seedlings were growing over the edge of the paper towels which may negatively affect the shoot and root measurements. Seedling root growth was significantly higher at 7 d compared to 5 d, maximising root growth and allowing more accurate measurements of traits. Therefore, the 7 d time point was selected for screening the effects of soaking the seeds in solutions containing PGRs.

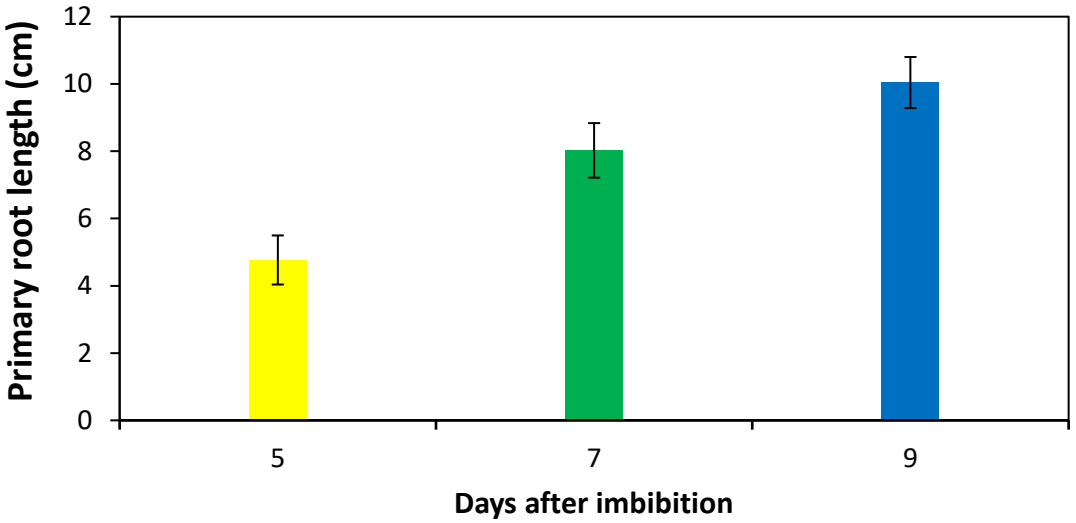


Figure 3.2. Primary root length (cm) of seedlings grown for 5, 7 and 9 d to optimise the time for seedlings to grow in the filter paper system and allow root and shoot traits to be measured accurately. Seeds were soaked for 24 hrs in deionised water and then placed on moist paper towels in an illuminated temperature-controlled incubator set to 34/11°C day/night. Data shown are means ± SEM (n=10).

3.4. The effect of soaking rice seeds in PGRs on root and shoot traits.

Seedling growth assays were conducted to investigate the effect of soaking IR64 rice seeds for 24 hours in different PGRs. The traits measured were, seedling fresh weight (FW; mg), seedling dry weight (DW; mg), DW/FW ratio, primary root length (cm), total root length (cm), lateral root count, average lateral root length (cm), shoot length (cm), aerial roots number and total shoot length with lateral (cm).

Gibberellin significantly ($P<0.001$) increased the primary root length and shoot length with increasing hormone concentration from 125 µM to 1000 µM (Table 3.1 and Figure 3.3). Primary root length and shoot length significantly decreased with increasing cytokinin (BA) concentrations, with primary roots being 50% shorter when treated with 1000 µM BA compared to the controls (Figure 3.4). Zeatin, also a cytokinin, was also tested. However, due to the requirement to dissolve zeatin in NaOH, the effect of NaOH on seedling germination was negative for the concentrations tested here (Appendix 1). Similarly, there was no significant differences ($P>0.05$) for treating seeds with 100, 250, 500 and 1000 µM Kinetin (Kin) for seedling fresh weight (mg), primary root length (cm), total root length (cm) and average lateral root length (Table 3.1). For auxin, there was no significant effect for the majority of traits, especially, for Indole-3-acetic acid (IAA). While 1-Naphthaleneacetic acid (NAA), significantly ($P<0.001$) increased primary root length, total root length, shoot length, total shoot length (Table 3.1 and Figure 3.5). However, this increase was optimum at the 250 µM for root and 500 µM for shoot with no significant difference ($P>0.001$) from 250 µM. Therefore, 250 µM was the optimization concentration for NAA in this study.

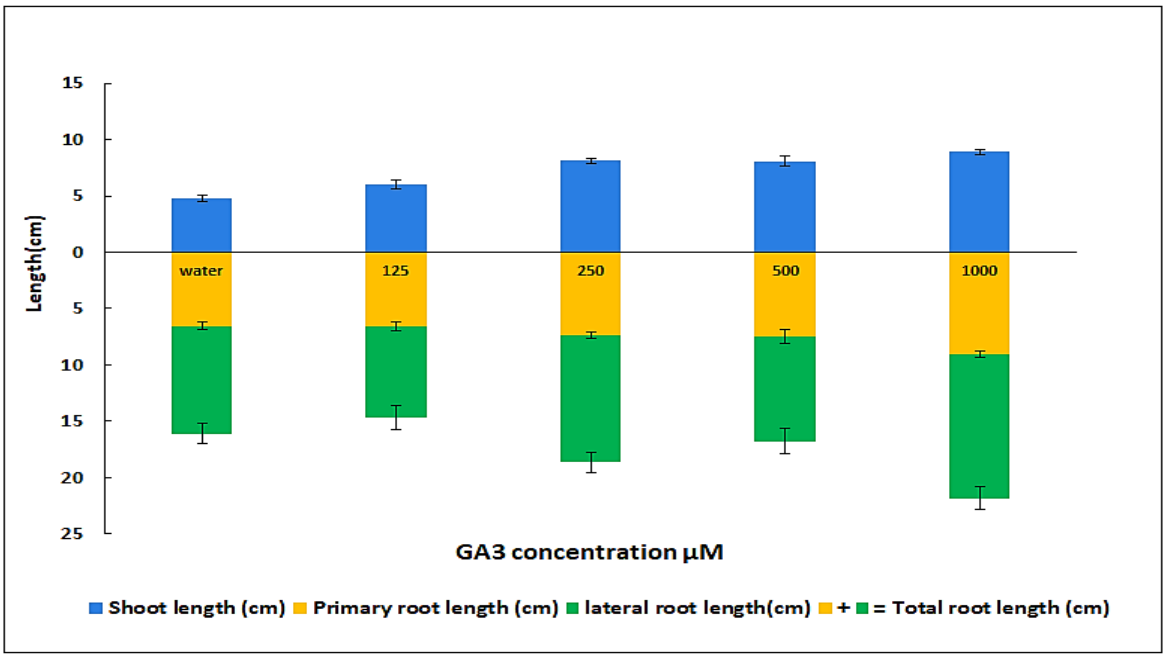


Figure 3.3. Shoot length (cm), primary root length (cm), lateral root length (cm) and total root length (cm) of IR64 rice seedlings grown for 7d. Seeds were soaked for 24 hrs in different concentrations of GA3 before being placed on moist paper towels and placed in an illuminated temperature-controlled incubator set to 34/11°C day/ night. Data shown are means ± SEM (n=10).

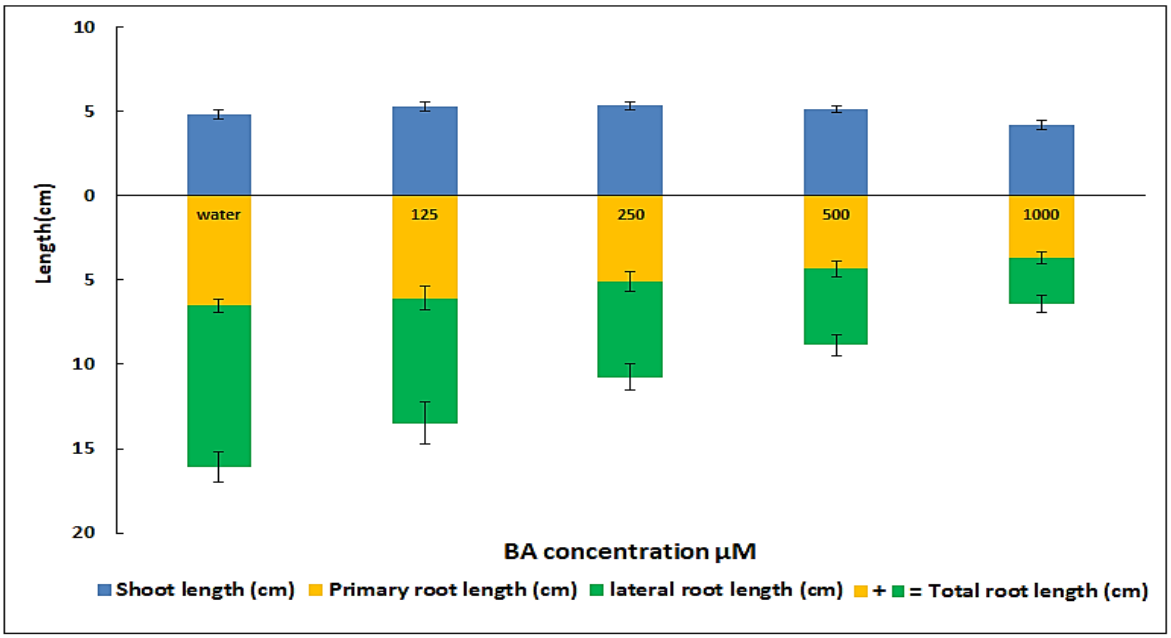


Figure 3.4. Shoot length (cm), primary root length (cm), lateral root length (cm) and total root length (cm) of IR64 rice seedlings grown for 7d. Seeds were soaked for 24 hrs in different concentrations of BA before being placed on moist paper towels and placed in an illuminated temperature-controlled incubator set to 34/11°C day/ night. Data shown are means ± SEM (n=10).

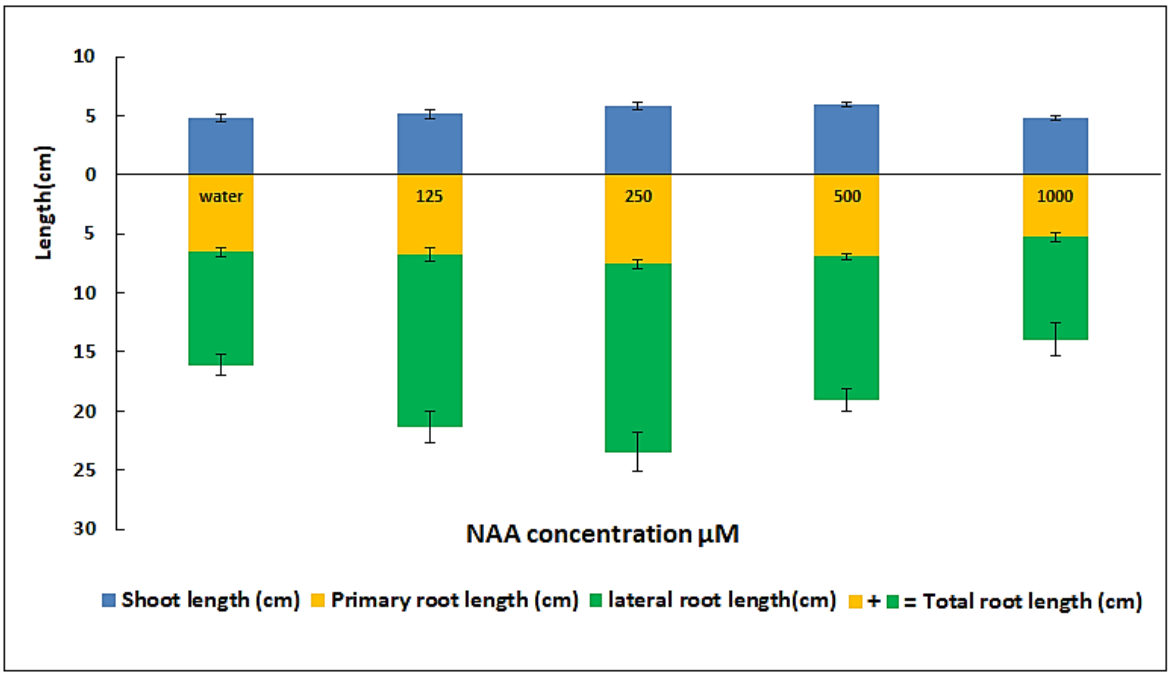


Figure 3.5. Shoot length (cm), primary root length (cm), lateral root length (cm) and total root length (cm) of IR64 rice seedlings grown for 7d. Seeds were soaked for 24 hrs in different concentrations of NAA before being placed on moist paper towels and placed in an illuminated temperature-controlled incubator set to 34/11°C day/ night. Data shown are means ± SEM (n=10).

Table 3.1. Summary of the main effect of different plant growth regulators on rice genotype IR64 root and shoot traits. Seedlings were allowed to grow for 7d before roots and shoots were measured. NS: refer to non-significant, P: refer to the P-value.

PGR	Traits									
	Seedling FW (mg)	Seedling DW (mg)	DW/FW ratio	Primary root length (cm)	Total Root Length (cm)	Lateral Root Count	Average lateral root length	Shoot length	Aerial roots number	Total Shoot length with lateral
IAA	NS	NS	NS	P =0.006	NS	NS	P=0.032	P=0.014	NS	P=0.020
NAA	NS	P=0.036	NS	P= 0.002	P=0.004	NS	P=0.037	P=0.010	NS	P=0.023
BA	P=0.03	P=0.043	NS	P <0.001	P<0.001	P<0.001	P=0.026	P=0.008	P<0.001	P<0.001
Kin	NS	P=0.038	NS	NS	NS	P= 0.001	NS	P=0.018	P= 0.004	P= 0.035
Zea	NS	P=0.023	NS	P=0.018	P=<0.001	P<0.001	NS	P=0.010	P=0.006	P=<0.001
GA3	P<0.001	P=0.023	P=0.002	P<0.001	P<0.001	P<0.001	NS	P<0.001	P=0.018	P<0.001

3.5. The effect of soaking the seeds of different rice genotypes in deionised water on primary root length, shoot length and total root length traits.

The seeds of twenty rice genotypes were soaked for 24 hours in distilled water to investigate the differences in root and shoot traits between the different genotypes before applying PGRs. There were significant differences ($P<0.001$) between the genotypes for the shoot and root lengths, total root lengths, lateral root lengths and numbers, primary root length and aerial root numbers (Figure 3.6). NSICRC9 produced the longest primary root length and shoot length compared with other genotypes (Figure 3.6). IR64 had the fourth lowest value for primary root length among the genotypes, with Daw Pao having the shortest primary root length. There was a nearly four-fold difference between the genotypes with the longest and shortest primary root lengths (Figure 3.6). Results also showed there were significant differences ($P<0.001$) between the genotypes for the shoot lengths. NSICRC9 had the greatest shoot length compared with the other genotypes, followed by AUS BAK TULSI, BAZIAN, AMBAR and IR64 (Figure 3.6). GHRAIBA 52 and KAOHSIUNG 136 had the shortest shoot lengths.

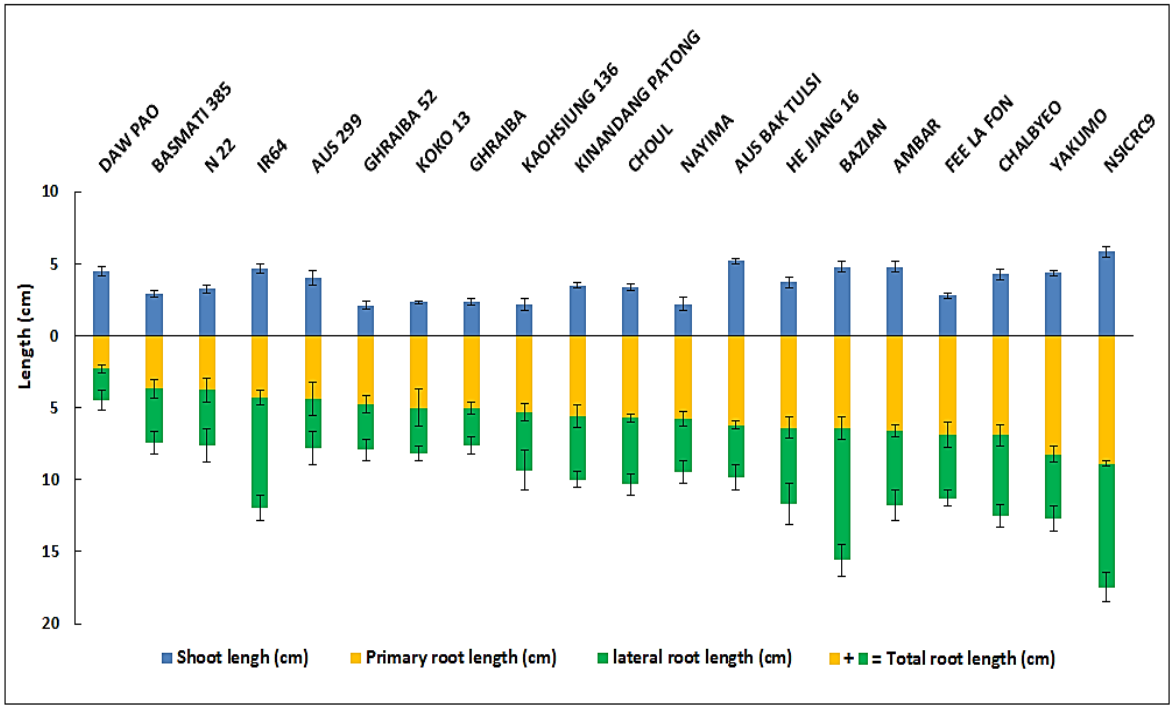


Figure 3.6. Variation in shoot length, primary root length, lateral root length and total root length among 20 different rice genotypes. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/ night before roots were measured. Data represent means ± SEM (n=30).

3.6. The effect of soaking the seeds of different rice genotypes in deionised water on aerial root count and lateral root count traits.

NSICRC9 also had the highest lateral root count, with an average of 50.25 lateral roots, followed by BAZIAN, AMBAR, CHALBYEO and IR64 (Figure 3.7). There was a nearly two-fold difference between the genotypes with the most and fewest numbers of lateral roots (Figure 3.7). The number of aerial roots varied significantly ($P<0.001$) between genotypes with YAKUMO, IR64, CHALBYEO, HE JIANG-16 and AMBAR having the highest numbers, respectively, compared with GHRAIBA, GHRAIBA-52 and NAYIMA with the lowest lateral root numbers (Figure 3.7).

3.7. The effect of soaking the seeds of different rice genotypes in deionised water on total root length.

As a consequence of having the longest primary root length and highest lateral root counts NSICRC9, BAZIAN, YAKUMO, CHALBYEO and AMBAR had the highest total root lengths (primary root + lateral root length) compared with lowest values for genotypes DAW PAO and BASMATI 385 (Figure 3.6). For the shoot system, genotypes with longest shoot and highest aerial roots number were CHALBYEO, YAKUMO, NSICRC9, BAZIAN and AMBAR. In contrast, genotypes with either lowest shoot length or lowest aerial root number or both of the two traits, were GHRAIBA 52, GHRAIBA and NAYIMA (Figure 3.6, 3.7).

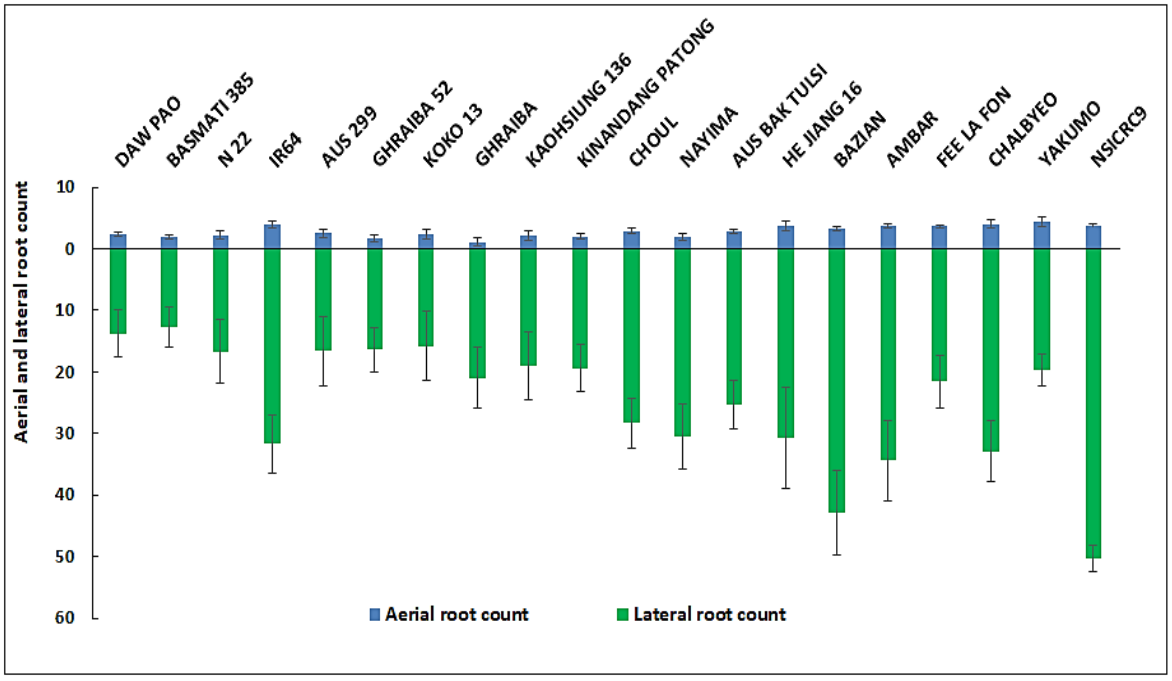


Figure 3.7. Variation in aerial root count and lateral root count among 20 different rice genotypes. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/ night before roots were measured. Data represent means ± SEM (n=30).

3.8. Analysis of soaking seeds of different rice genotypes in different PGRs

The seeds of twenty rice genotypes were soaked for 24 hours in 1000 µM GA3, 3000 µM GA3 and 250 µM NAA, based on the response of IR64 to these treatments, to investigate the variation in root and shoot traits to the different PGRs. All data from all treatments (35,071 data points) were subject to correlation analysis and Principal Component Analysis (PCA) to investigate relationships between the different traits. Correlation analysis was used to investigate correlations between individual traits across the experiment. All correlations were significant (P<0.001) and positive. As expected, primary root length, lateral root length and lateral root count were similarly and strongly correlated to total root length with r values of 0.8 (Figure 3.8).

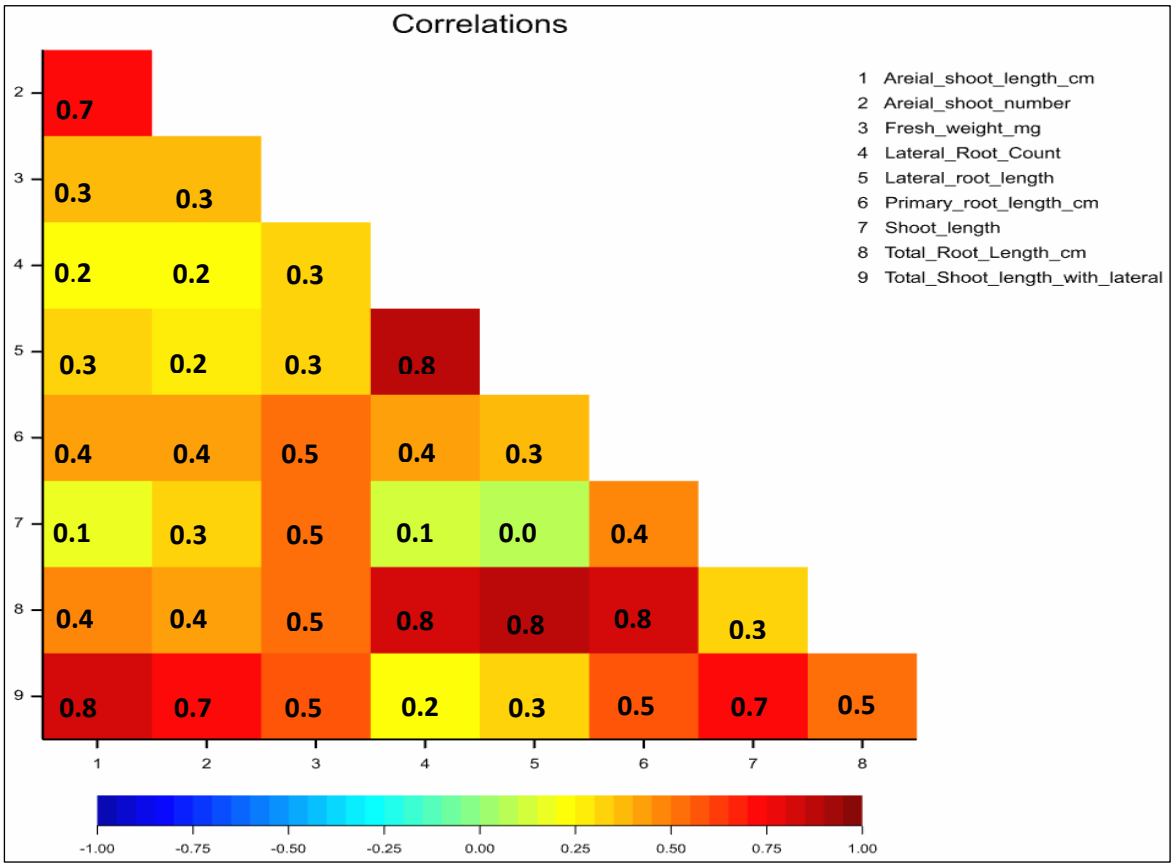


Figure 3.8. Correlation coefficient values between all shoot and root traits which have been presented in this study. Colours indicate to the strength of the relationship between two variables. The darker red colour refers to stronger statistical relationship between variants, were correlations significant at p<0.001. Data represent the mean of 12 seedlings per treatment (n=36).

This highlights the significance of lateral root length and lateral root count in the contribution to total root length. This could be an important trait for breeders when selecting genotypes for root system breeding programme. In addition to this, obtaining long efficient lateral roots with a long primary root length results in a vigorous root system which could enhance drought tolerance under arid and semi-arid conditions. In contrast, total shoot length was correlated positively to aerial shoot length and aerial shoot number with 0.8 and 0.7 respectively (Figure 3.8).

Analysis of the data using PCA showed that Principal Component 1 (PC1) and PC2 accounted for 72.58% of the variation in the dataset. Latent vector loadings for lateral root traits explained variation associated with PC1 and latent vector loadings for shoot traits explained variation in PC2 (Figure 3.9). Strong positive correlation among total root length (cm), lateral root count, and lateral root length (cm) was observed within PC1. In contrast, shoot length (cm), aerial shoot number and total shoot length with lateral (cm) were correlated positively within PC2 (Figure 3.9).

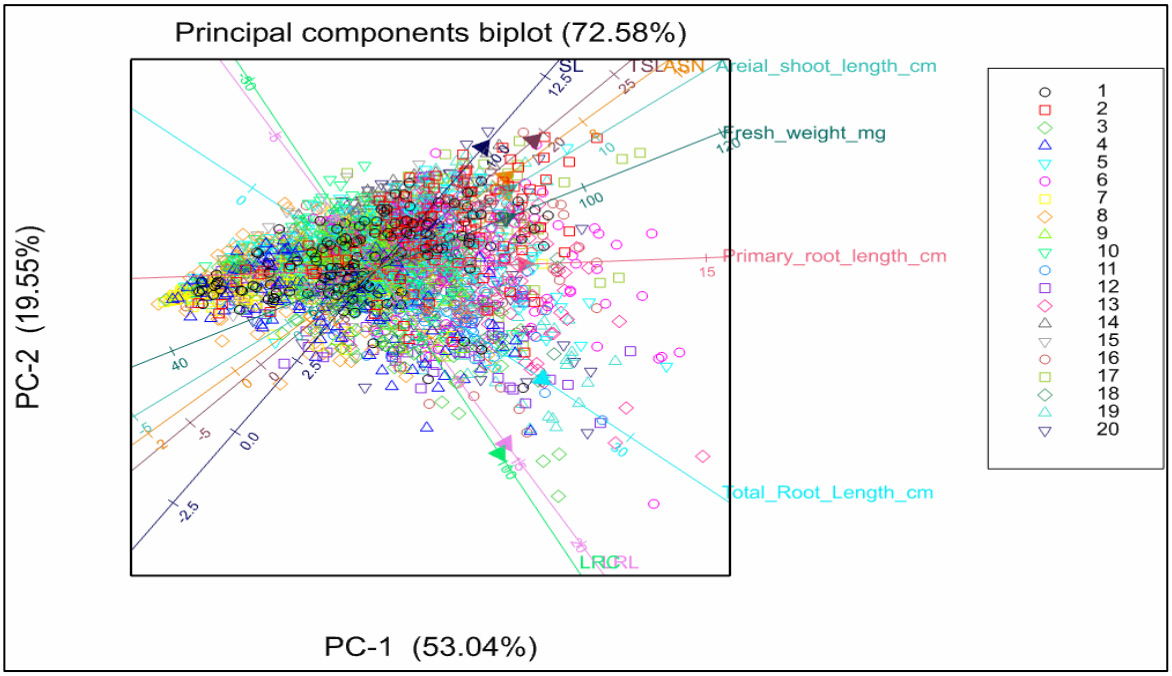


Figure 3.9. Principal Component analysis (PCA) of shoot and root traits for 20 different genotypes treated with different PGRs. Data represent (35,071 points). Numbers 1-20 represent genotypes (Table 2.1) and from top figure (SL) is abbreviation for Shoot length; (TSL) is abbreviation for Total Shoot length with lateral; (ASN) is abbreviation for Aerial shoot number; (LRC) is abbreviation for Lateral Root Count and (LRL) is abbreviation for Lateral root length.

3.9. The effect of soaking seeds of different rice genotypes in 3000 µM GA3 on root traits.

Soaking rice seeds in 3000 µM GA3 significantly (P<0.001) increased the primary root length for GHRAIBA, GHRAIBA52, NAYIMA, CHOUL, YAKUMO, HE JIANG 16 and AUS 299 compared with their non-treated controls. While for GHRAIBA, GHRAIBA52, NAYIMA and CHOUL 3000 µM GA3 significantly (P<0.001) increased the primary root length by 100%, 56.9%, 27.8%, 24.1% compared with their controls, respectively. For some genotypes, soaking seeds in 3000 µM GA3 either did not affect primary root length or reduced it compared to their controls, for example DAW PAO and AMBAR, respectively (Figure 3.10).

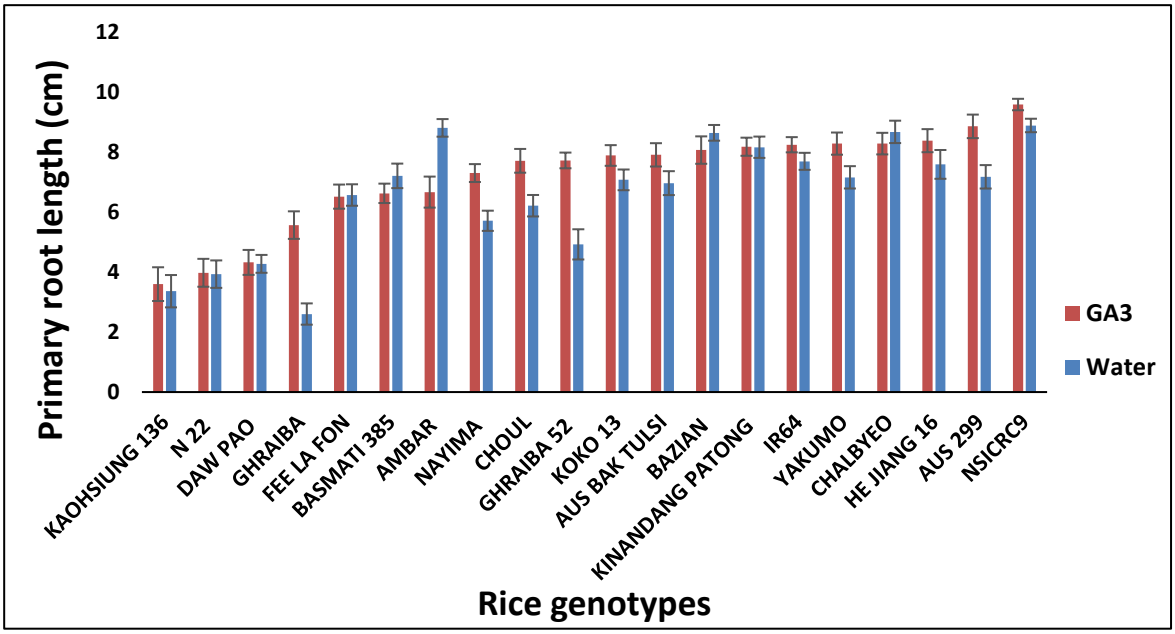


Figure 3.10. Primary root length (cm) of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 3000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

For lateral root count, a positive effect of treatment was higher for only one genotype, GHRAIBA, compared with the non-treated controls (Figure 3.11), while 3000 µM GA3 negatively ($P<0.001$) affected lateral root counts for all other genotypes (Figure 3.11). Meanwhile, 3000 µM GA3 negatively ($P<0.001$) affected lateral root length for all genotypes (Figure 3.12).

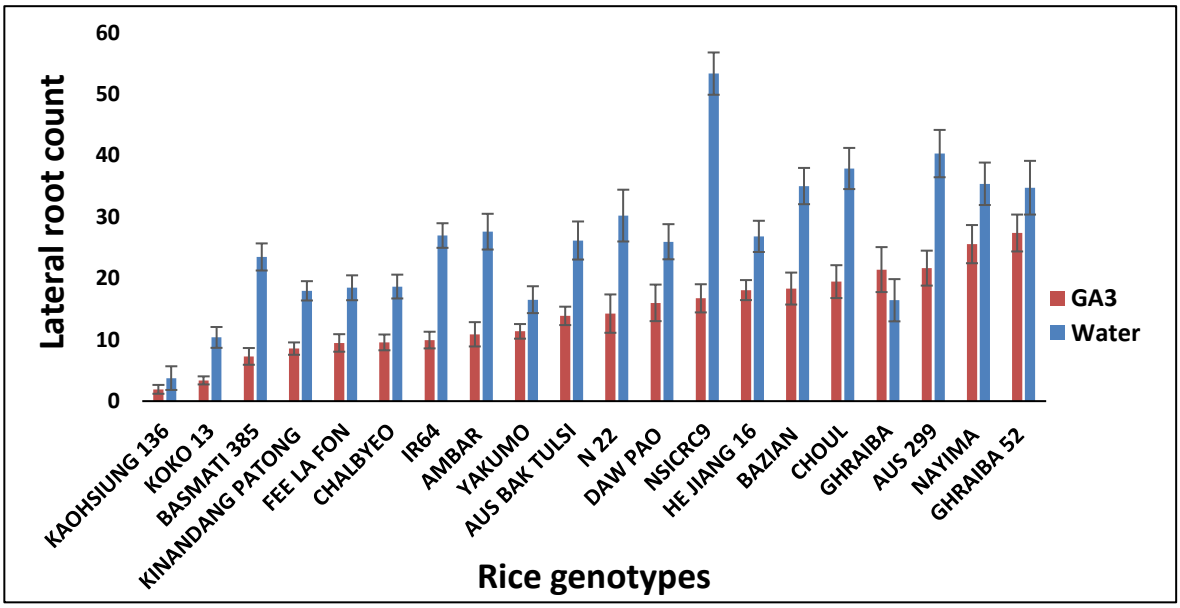


Figure 3.11. Lateral root count of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 3000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

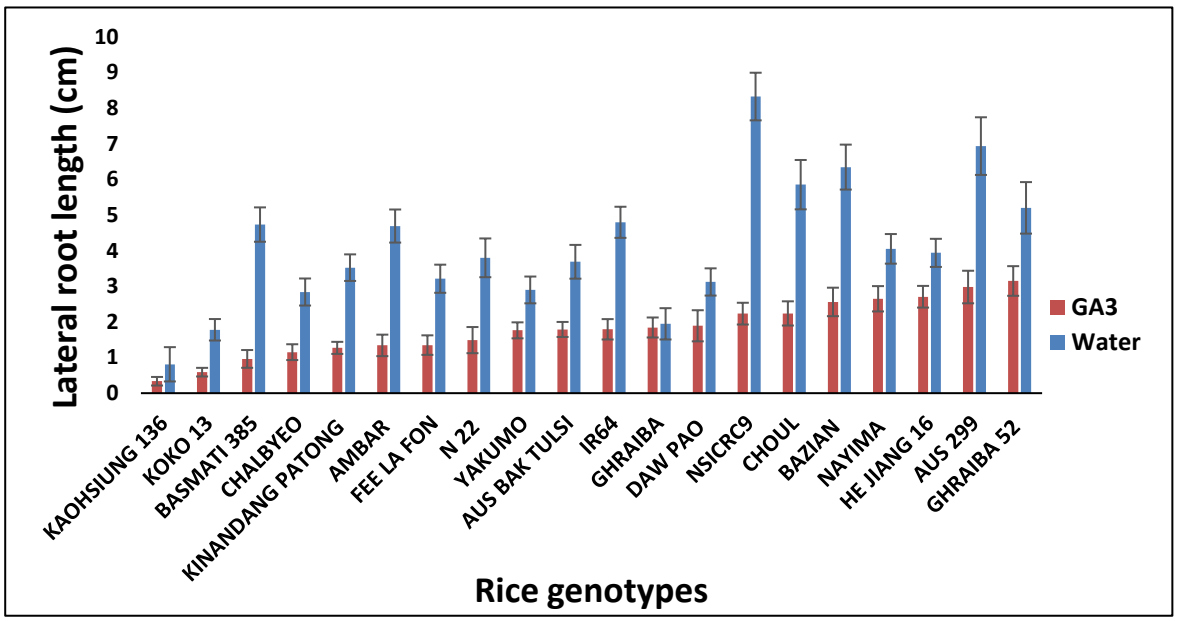


Figure 3.12. Lateral root length (cm) of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 3000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

For total root length, GHRAIBA was again the only genotype that responded positively and significantly (P<0.001) to its seeds being soaked in 3000 µM GA3 compared with the control. However, total root length of all other genotypes showed either no significant difference or were significantly (P<0.001) shorter total root lengths compared to their controls (Figure 3.13).

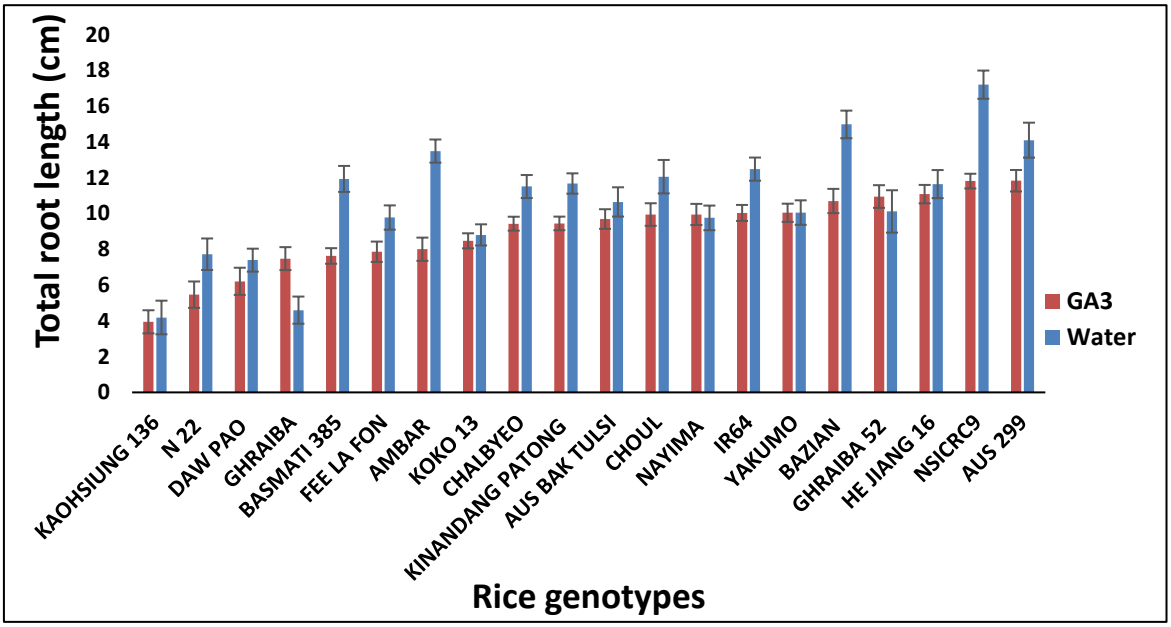


Figure 3.13. Total root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 3000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

3.10. The effect of soaking seeds of different rice genotypes in 3000 µM GA3 on shoot traits.

The effect of soaking seeds in 3000 µM GA3 significantly (P<0.001) affected shoot length for all genotypes except KAOHSIUNG 136 (Figure 3.14). Seedlings of NSICRC9, IR64, AUS 299 and AMBAR treated with 3000 µM GA3 had the highest shoot lengths, whilst seedlings of KAOHSIUNG 136, GHRAIBA, N22 and FEE LA FON treated with 3000 µM GA3 had the lowest shoot lengths (Figure 3.14). However, for aerial root length there was a negative response of some genotypes to 3000 µM GA3 whereas there was no response of other genotypes (Figure 3.15).

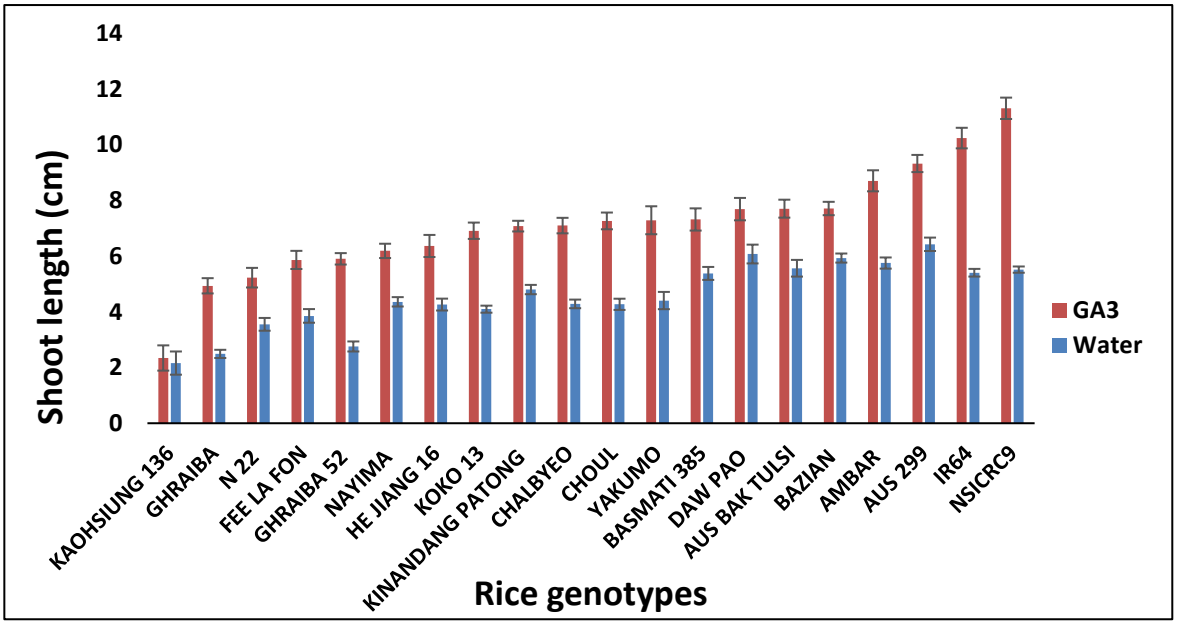


Figure 3.14. Shoot length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 3000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before shoots were measured. Data shown are means ± SEM (n=36).

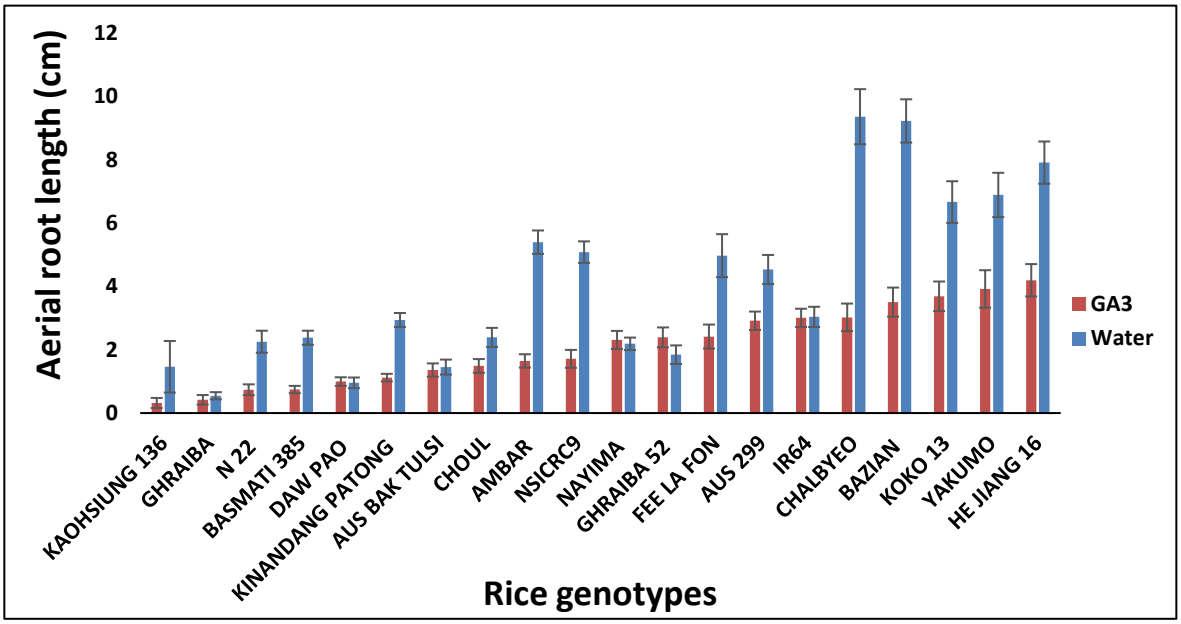


Figure 3.15. Aerial root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 3000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before shoots were measured. Data shown are means ± SEM (n=36).

3.11. The effect of soaking seeds of different rice genotypes in 1000 µM GA3 on root traits.

When seeds were soaked in 1000 µM GA3 there were just five genotypes showing a significant (P<0.001) increase in primary root length among the twenty genotypes; N22, GHRAIBA52, GHRAIBA, CHOUL and AUS 299. Genotype GHRAIBA showed the largest increase in primary root length (212% increase) compared with its control. This increase in the primary root length moved the GHRAIBA genotype from the genotype with the shortest primary root length to 7th out of 20, with no significant (P<0.001) difference with CHOUL, AMBAR, HE JIANG 16 and BAZIAN genotypes (Figure 3.16).

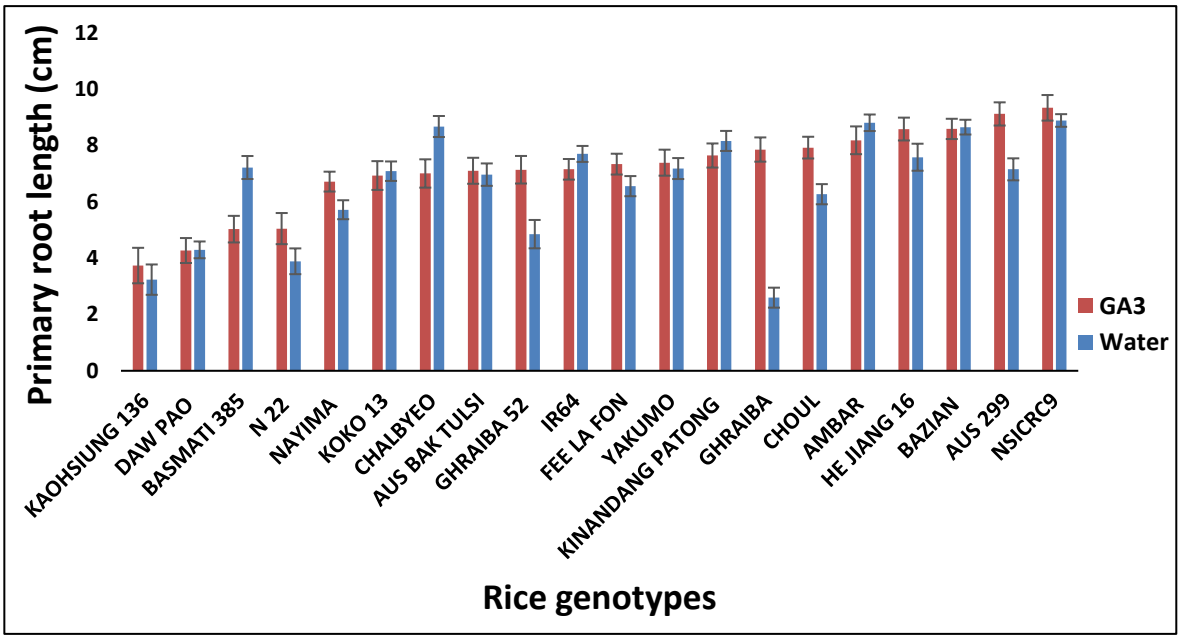


Figure 3.16. Primary root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 1000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

For lateral root count, lateral root length and total root length GHRAIBA was the only genotype that responded significantly (P<0.001) to soaking its seeds in 1000 µM GA3 treatment. GHRAIBA increased its total root length by 140.13% when its seeds were soaked in 1000 µM GA3 compared with its control.

All other genotypes showed either no significant difference, such as KAOHSIUNG 136, YAKUMO and HE JIANG 16 or had significantly (P<0.001) lower lateral root count, shorter lateral root length and shorter total root lengths, compared to their controls such as BASMATI 385, CHALBYEO, BAZIAN and NSICRC9 (Figures 3.17; 3.18; 3.19).

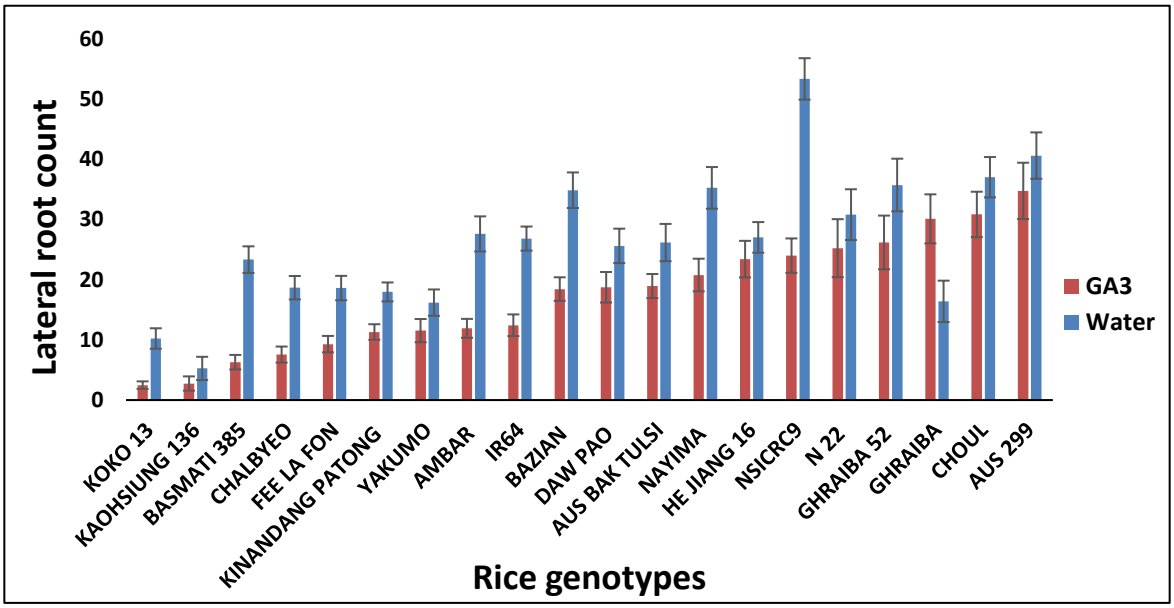


Figure 3.17. Lateral root count of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 1000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

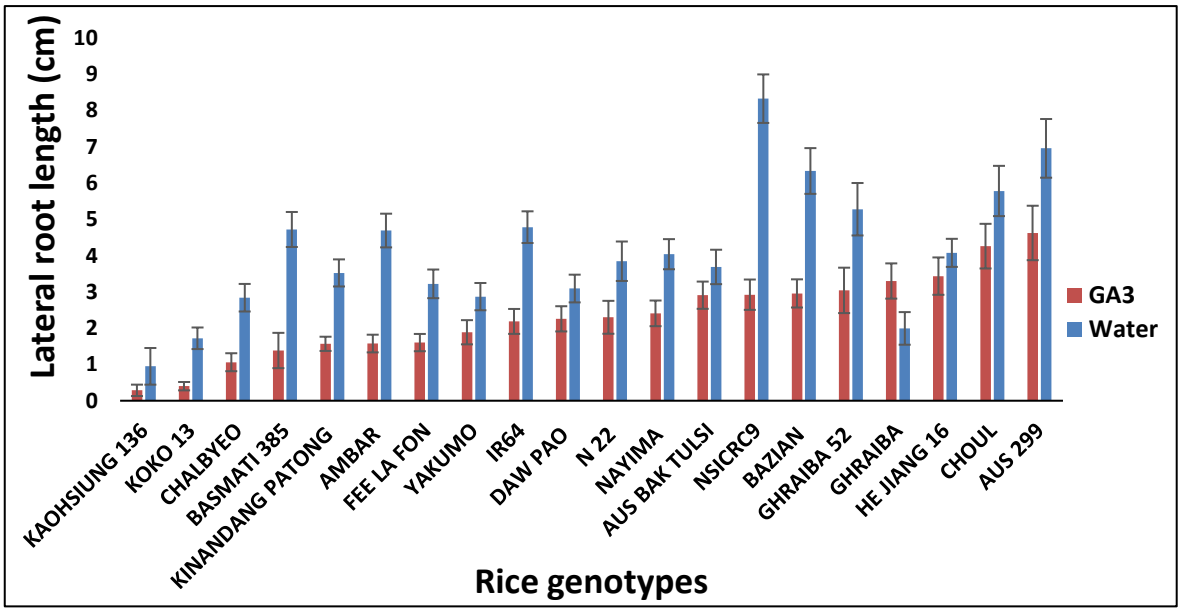


Figure 3.18. Lateral root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 1000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

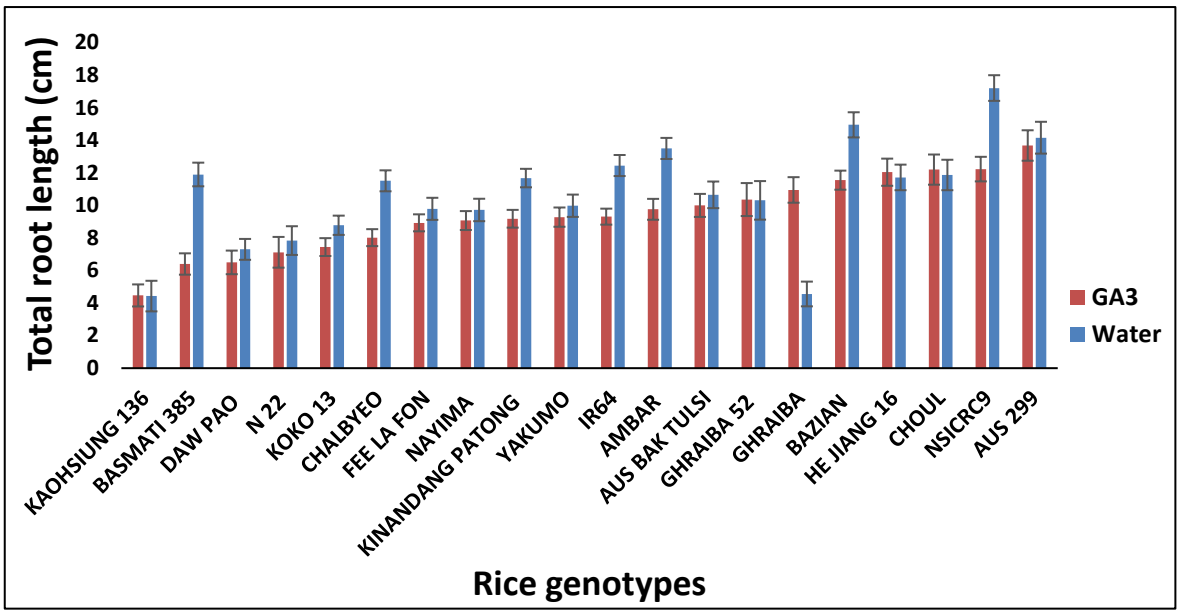


Figure 3.19. Total root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 1000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

3.12. The effect of soaking seeds of different rice genotypes in 1000 µM GA3 on shoot traits.

The effect of soaking seeds in 1000 µM GA3 significantly ($P<0.001$) increased the shoot length for most of genotypes except KAOHSIUNG 136 (Figure 3.20). Seedlings of NSICRC9, IR64, AUS 299, AMBAR and DAW PAO treated with 1000 µM GA3 had the longest shoot lengths with an average of 9.3, 9.2, 8.7, 8.4 and 8.0 cm, respectively, compared with controls which had 5.5, 5.3, 6.4, 5.7 and 6.0 cm, respectively. Seedlings of KAOHSIUNG 136 treated with 1000 µM GA3 had the shortest shoot lengths (Figure 3.20).

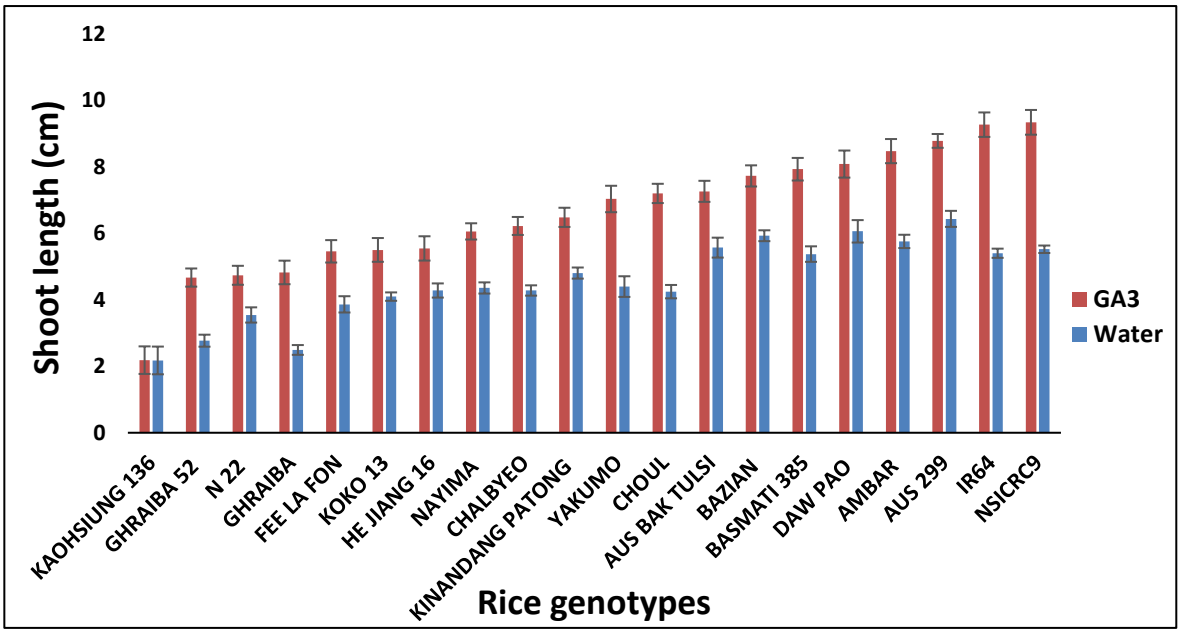


Figure 3.20. Shoot length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 1000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

DAW PAO was the only genotype where soaking its seeds in 1000 µM GA3 significantly ($P<0.001$) increased aerial root number compared with its control. All other 19 genotypes showed either no significant difference such as GHRAIBA, KINANDANG PATONG, IR64 and BAZIAN or had a significantly ($P<0.001$) lower aerial root number compared with their control such as NSICRC9, KOKO 13 and CHALBYEO (Figure 3.21). Consequently, soaking seeds in 1000 µM GA3 did not significantly increase aerial root length in any genotype, but it was significantly lower in several genotypes (Figure 3.22).

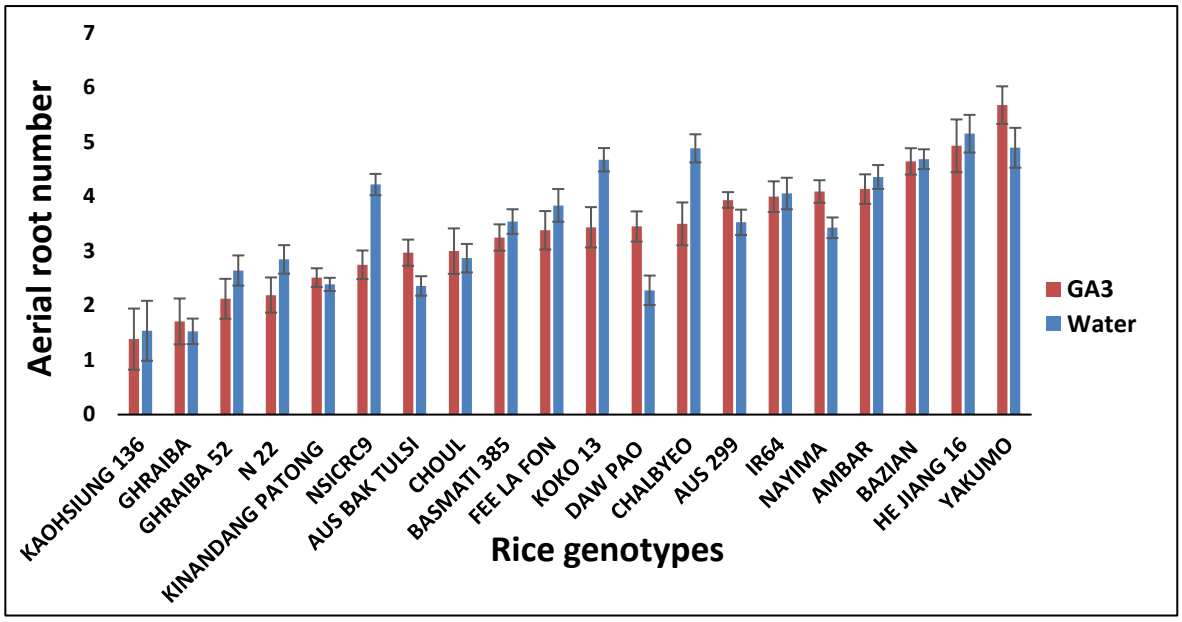


Figure 3.21. Aerial root number of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 1000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

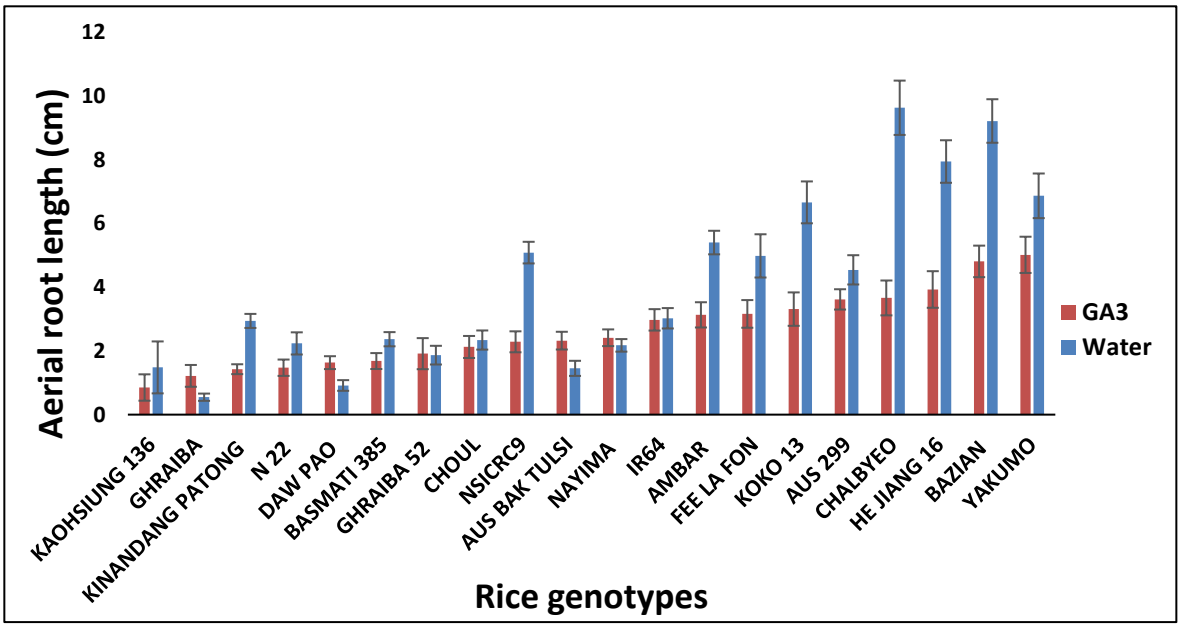


Figure 3.22. Aerial root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 1000 µM GA3 prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

3.13. The effect of soaking seeds of different rice genotypes in 250 µM NAA on root traits.

Treating seeds with 250 µM NAA significantly altered some root traits for some genotypes. GHRAIBA, GHRAIBA 52 and AUS 299, treated with 250 µM NAA had the longest primary root lengths which were 6.5 cm, 6.7 cm and 9.4 cm. These were significantly ($P<0.001$) longer than their non-treated controls which were 2.5 cm, 4.8 cm and 7.1 cm, respectively (Figure 3.23). However, the majority of genotypes either responded negatively to this concentration or had no effect on primary root length compared with their control (Figure 3.23).

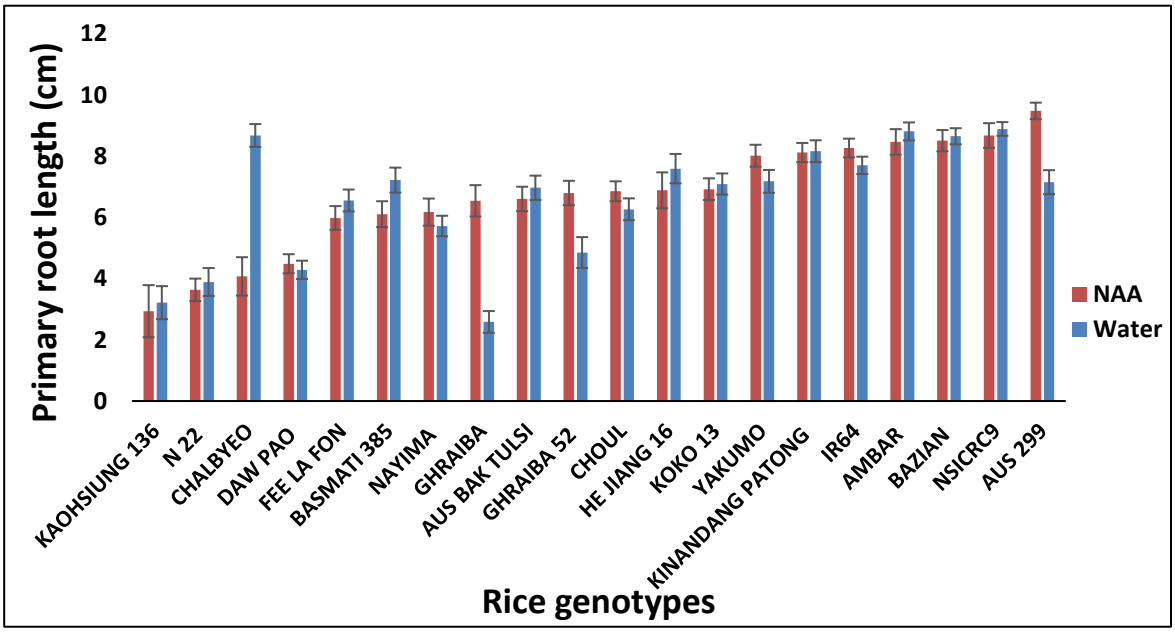


Figure 3.23. Primary root length (cm) of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 250 µM NAA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

The lateral root count of three genotypes, IR64, GHRAIBA and GHRAIBA 52, whose seeds were treated with 250 µM NAA were significantly (P<0.001) higher with 37.9, 38.7 and 44.2 lateral roots compared with the controls which had 27, 16.4 and 34.6, respectively (Figure 3.24). The lateral root lengths of the same three genotypes were significantly (P<0.001) greater with 4.7, 6.4 and 7.2 cm for GHRAIBA, GHRAIBA 52 and IR64 respectively, compared with their controls 1.9, 5.1 and 4.8, respectively (Figure 3.25).

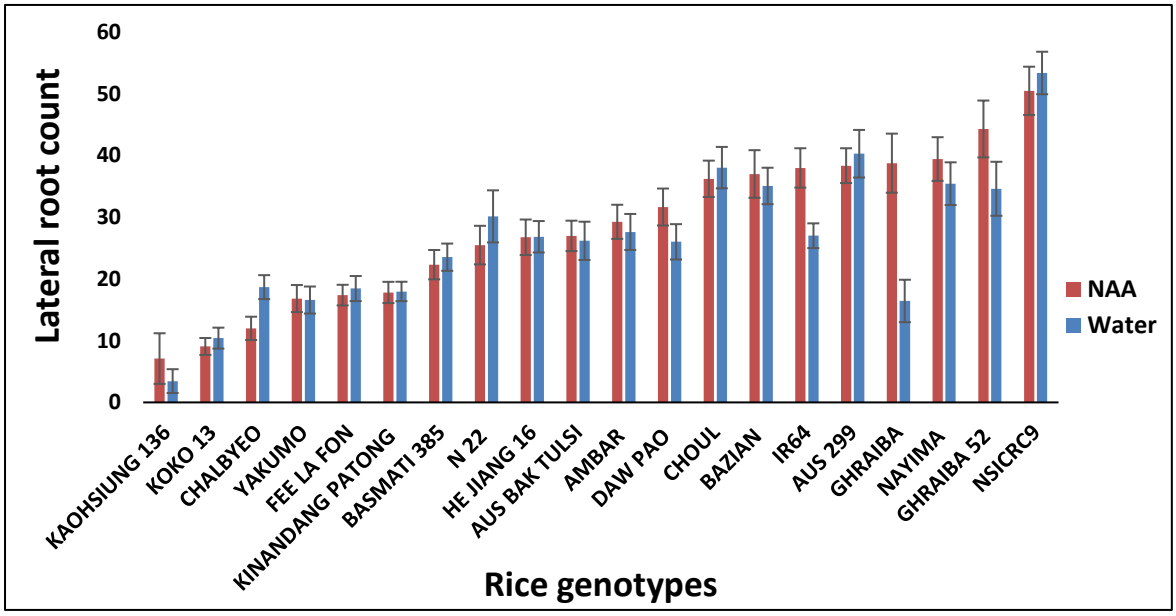


Figure 3.24. Lateral root count of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 250 µM NAA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

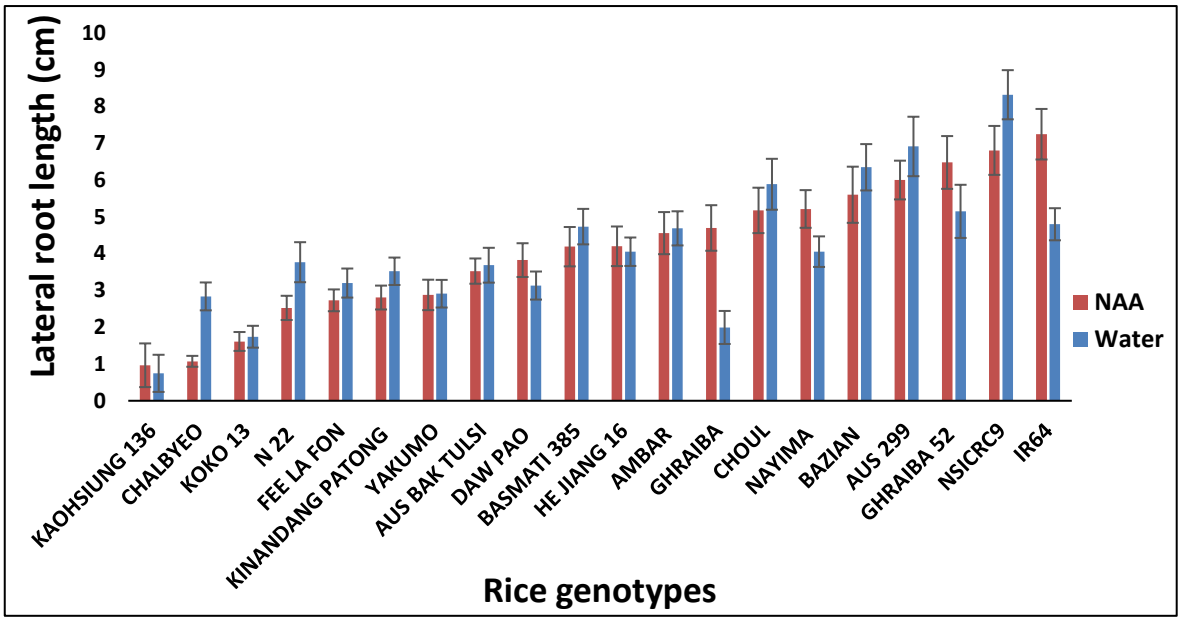


Figure 3.25. Lateral root length (cm) of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 250 µM NAA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

Resulting from having high number of lateral root count and long lateral roots IR64, GHRAIBA 52 and GHRAIBA seeds treated with 250 µM NAA had the longest total root lengths, which were 15.2 cm, 13.2 cm and 11.2cm compared with their controls, respectively. These were significantly ($P<0.001$) longer than their non-treated controls. While KAOHSIUNG 136, CHALBYEO and N22, whose seeds were treated with 250 µM NAA, had the shortest total root lengths for this trait with 3.9 cm, 5.1 cm, and 6.1 cm respectively, compared with controls and other genotypes (Figure 3.26). Although, the effect of treating seeds of IR64 with 250 µM NAA on primary root length was not significant (Figure 3.23), the high number of lateral roots (Figure 3.24) and higher average lateral root length resulted in the longest total root system, which was significantly ($P<0.001$) longer than its non-treated control (Figure 3.26). This explains the crucial role for the lateral root to increase the total root length of the genotypes.

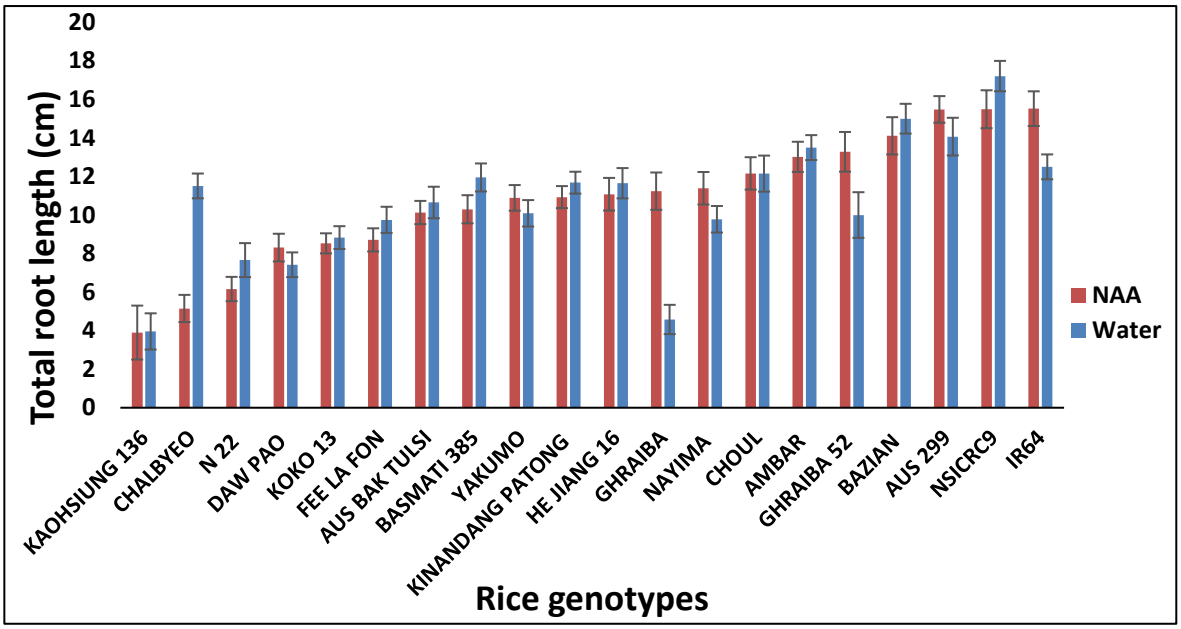


Figure 3.26. Total root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 250 µM NAA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

3.14. The effect of soaking seeds of different rice genotypes in 250 µM NAA on shoot traits.

Analysis of variance showed there were significant difference between the genotypes (P<0.001) as well as a significant (P<0.001) interaction between genotypes and treatment and there was no significant effect of soaking the seeds 250 µM NAA treatment. Thus, the shoot length for seedlings whose seeds were treated with 250 µM NAA were significant (P<0.001) longer than their controls for only two of the genotypes. The shoot lengths of GHRAIBA 52 and AUS 299 were 3.6 cm and 7.0 cm for the treated seeds compared with their controls which were 2.7 cm and 6.4 cm, respectively (Figure 3.27). Furthermore, AUS 299 genotype had the highest shoot length with the 7.0 cm compared with KAOHSIUNG 136 which had the lowest shoot length with only 1.9 cm for treated seeds with 250 µM NAA (Figure 3.27).

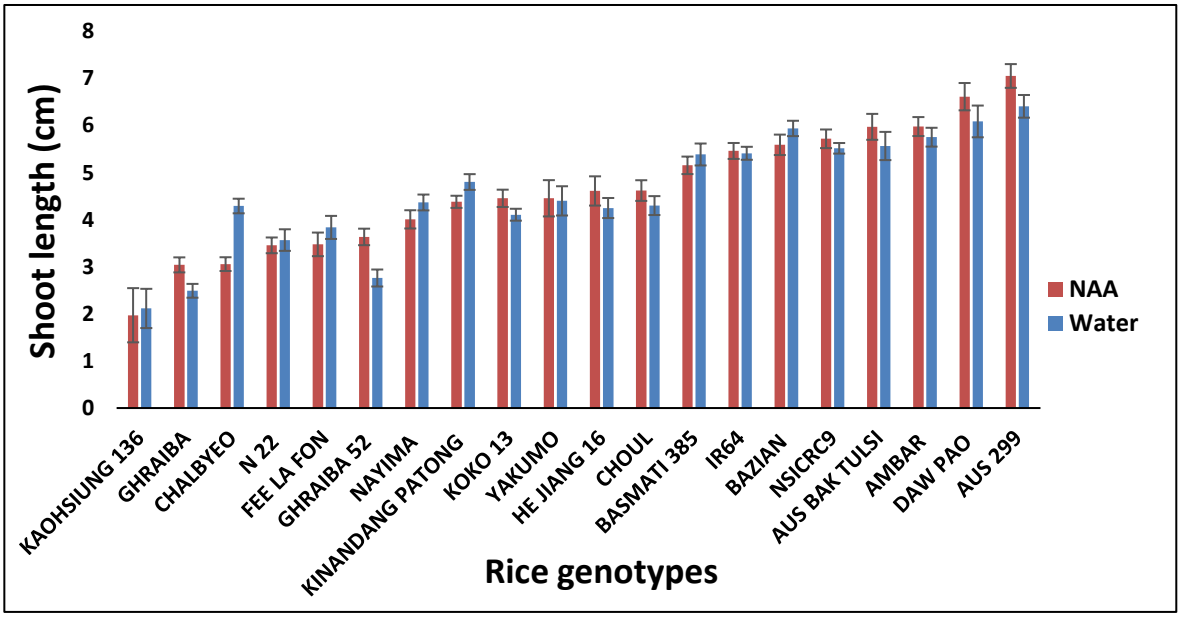


Figure 3.27. Shoot length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 250 µM NAA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

For aerial root number and aerial root length there were an interaction between GHRAIBA, GHRAIBA 52 and DAW PAO and 250 µM NAA, which showed significant ($P<0.001$) differences compared with their controls. Consequently, there were no overall significant ($P<0.001$) response in aerial root number and aerial root length for all genotypes for this treatment (Figures 3.28; 3.29).

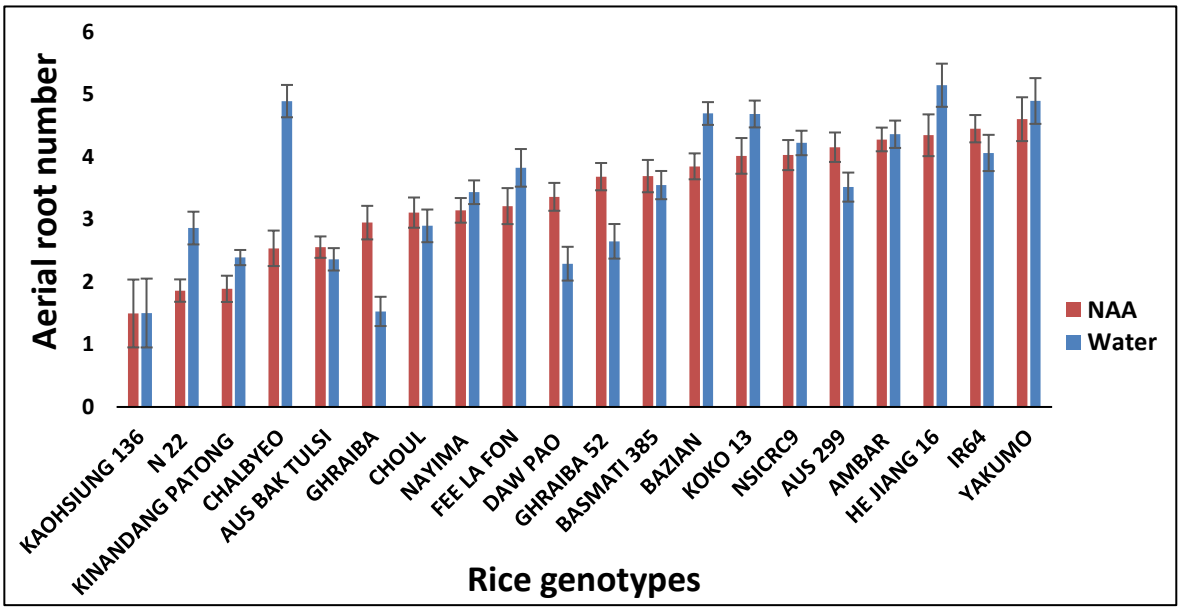


Figure 3.28. Aerial root number of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 250 µM NAA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

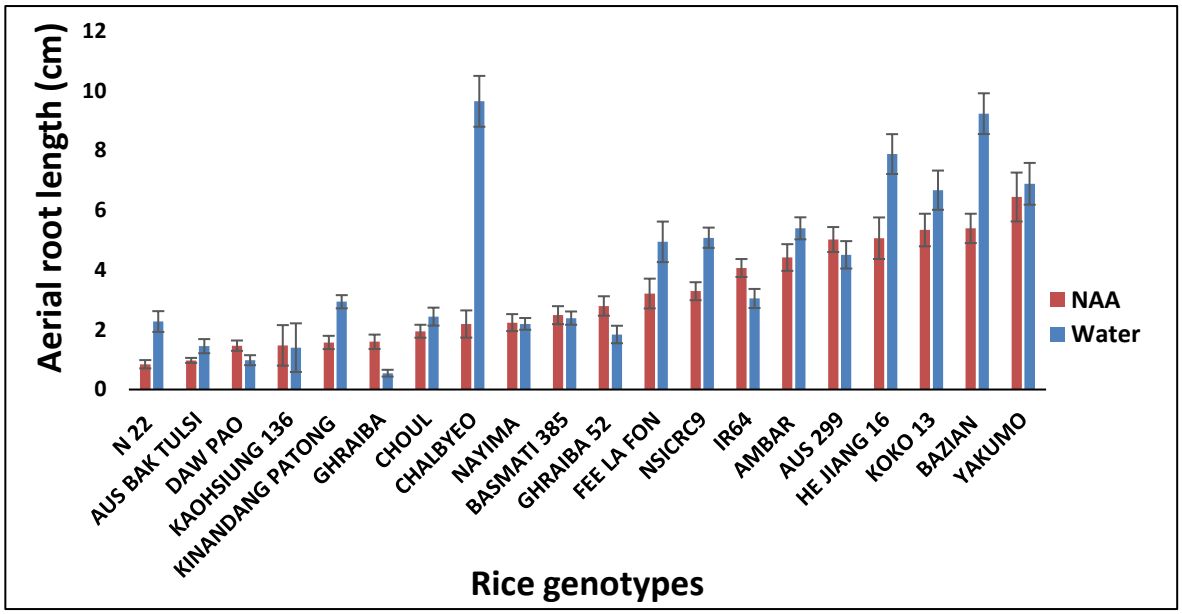


Figure 3.29. Aerial shoot length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 250 µM NAA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

3.15. The effect of soaking seeds of different rice genotypes in 125 µM BA on root and shoot traits.

Seedlings whose seeds were treated with 125 µM BA had significantly (P<0.001) shorter primary root lengths (Figure 3.30), lower lateral root counts (Figure 3.31) and shorter total root lengths (Figure 3.32) for all the genotypes compared to their controls.

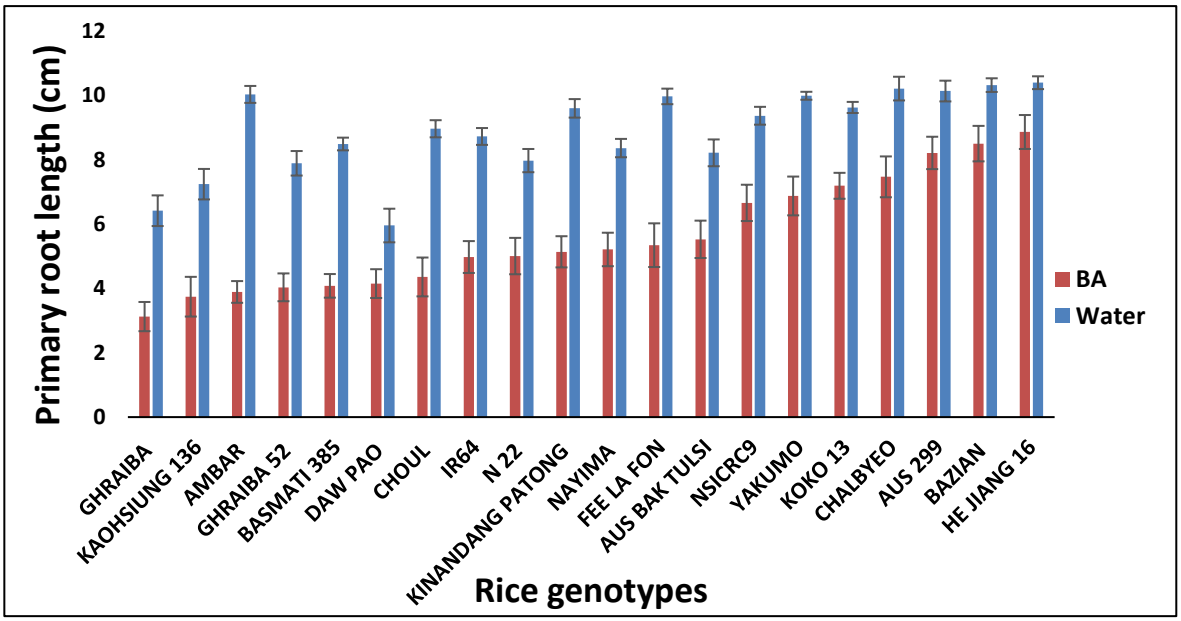


Figure 3.30. Primary root length (cm) of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 125 µM BA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

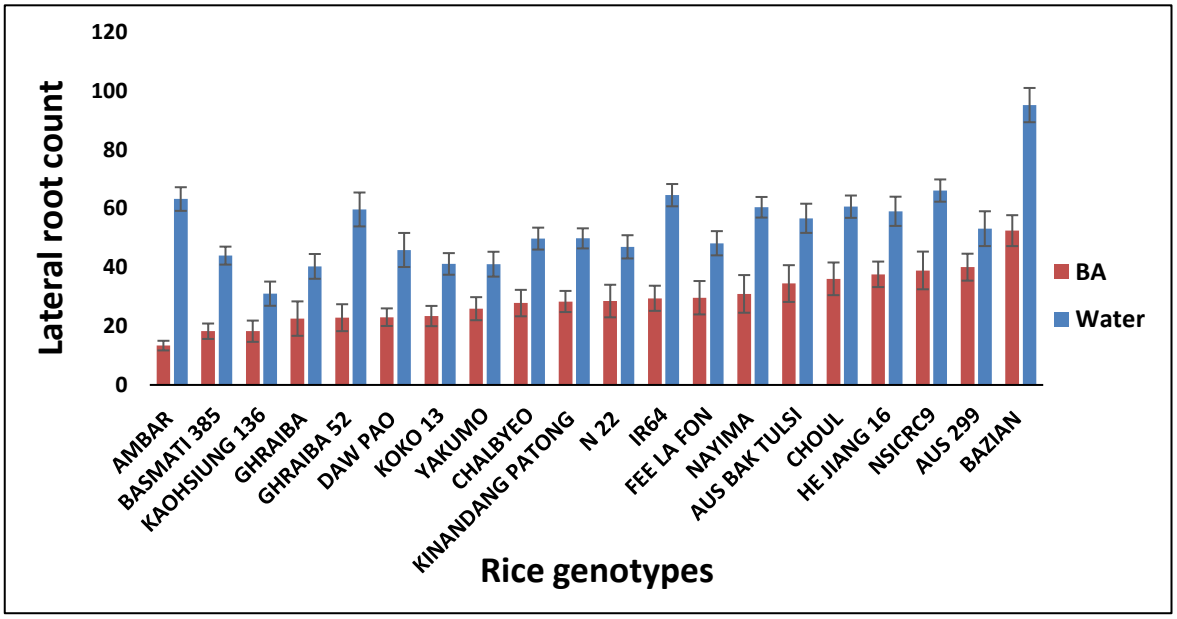


Figure 3.31. Lateral root count of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 125 µM BA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

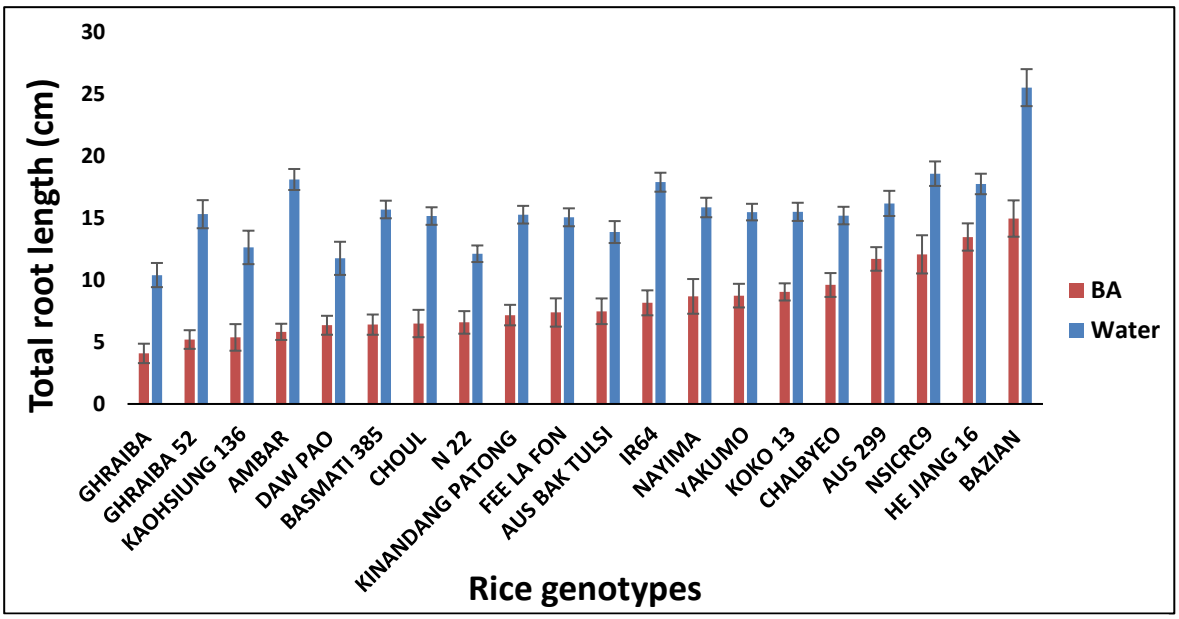


Figure 3.32. Total root length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 125 µM BA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

For Shoot length and aerial root number treated seeds with 125 µM BA either had a significant negative effect ($P<0.001$) or there were no significant ($P>0.05$) difference with their controls (Figures 3.33; 3.34).

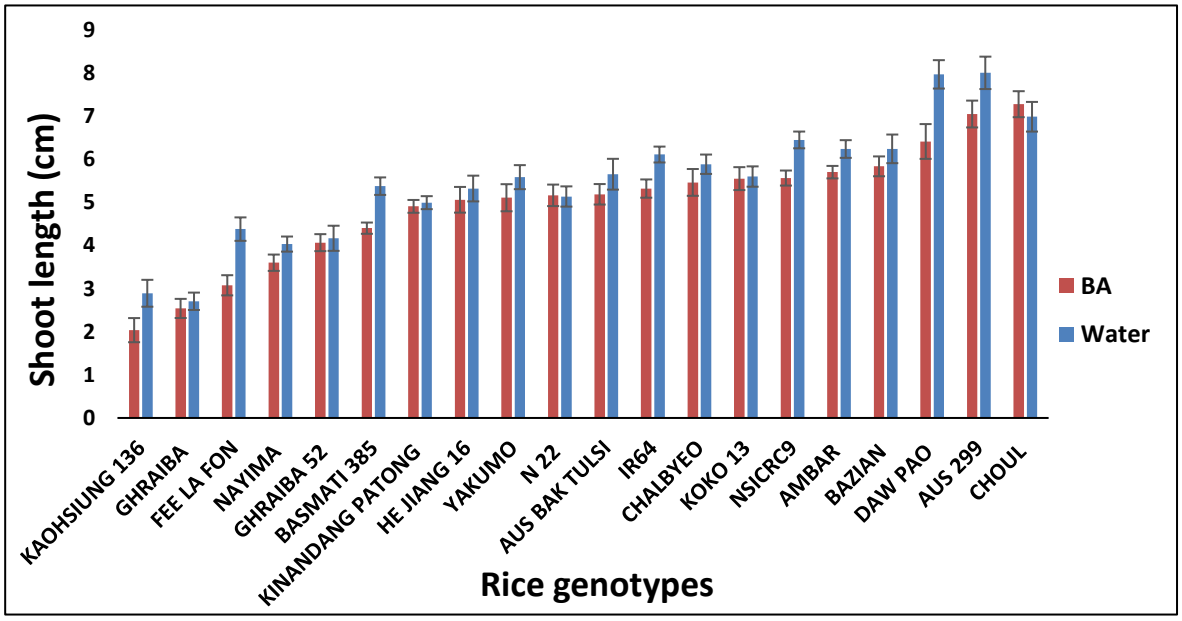


Figure 3.33. Shoot length of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 125 µM BA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

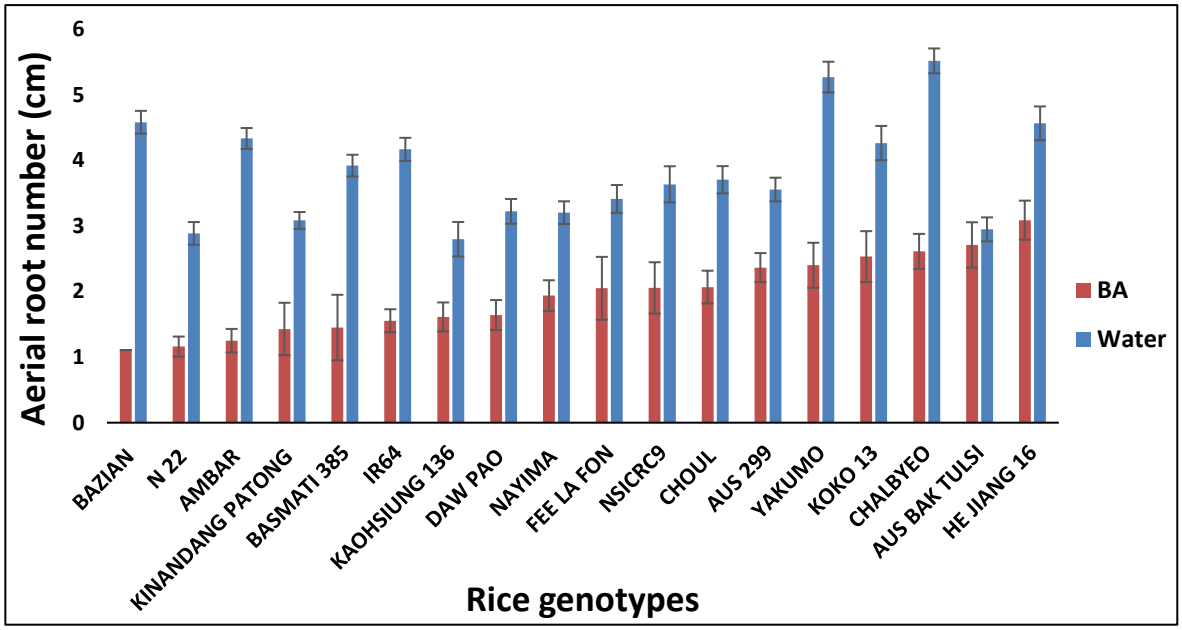


Figure 3.34. Aerial root number of seedlings grown for 7 d. Seeds were soaked for 24 hrs in 125 µM BA prior to germination on filter paper. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/night before roots were measured. Data shown are means ± SEM (n=36).

3.16. Correlation between two water control treatments for 20 different rice genotypes after soaking seeds in deionised water on shoot and root traits.

To ensure consistency between experimental runs, correlation analysis between the initial experiment with 20 genotypes and the water control treatment of the following experiment showed that these traits had similar responses across the genotypes in the experimental system. Genotypes with high primary root length, shoot length, lateral root count, lateral root length, total root length and shoot length maintain their positions across experiments (Figure 3.35). This demonstrates that the experimental system is robust, and data can be compared between experiments.

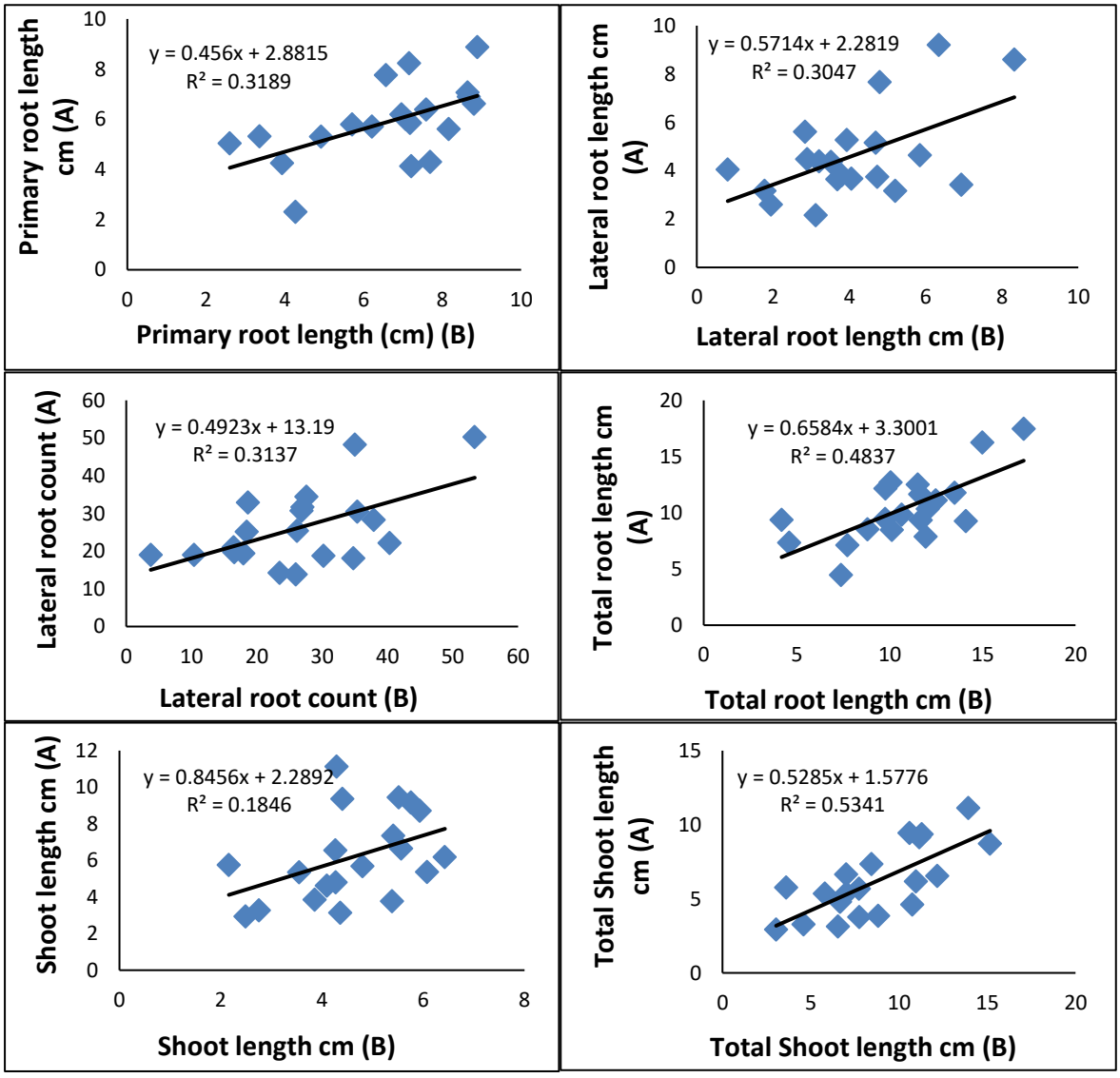


Figure 3.35. Correlation between root and shoot traits among 20 different rice genotypes. Seedlings were allowed to grow for 7 d in an illuminated temperature-controlled incubator set to 34/11°C day/ night before roots were measured. Data represent means ± SEM (n=30). Experiment (A) represent data for water within PGRs. Experiment (B) represent data for individual water experiment.

Discussion:

Results confirmed the ability of PGRs to modify the root and shoot growth and architecture in rice seedlings, with both positive and negative impacts on the traits measured (Figures 3.3-3.5; Table 3.1). The increase in primary root length during the early stages of the plant growth and establishing a longer root system, which in some genotypes was up to 4 cm longer (Figures 3.6 and 3.10), may impact on the future drought tolerance of these genotypes.

Gibberellins:

Treatment of seeds with GA had variable effects on root and shoot traits among rice genotypes. Results showed that soaking seeds in 3000 and 1000 µM GA3 positively $P<0.001$ affected shoot length more than root length for all genotypes (Figures 3.14 and 3.20). Since GA controls shoot development and mutations in its biosynthesis result in dwarf plants, a positive effect on shoot length was expected (Kanno *et al.*, 2016). NSICRC9 and IR64 had the longest shoot length compared with their controls and in comparison, to all other genotypes after treatment with 3000 µM GA3 (Figure 3.14). This increase in shoot length for IR64 after GA3 was applied could be explained through reducing the effect of the *sd1* mutation in IR64, which causes reduced active GA in the plant (Spielmeyer *et al.*, 2002). All other genotypes showed increases in shoot length following treatment of their seeds with 3000 µM GA, however it was not possible to determine if any of these genotypes also carried the *sd1* mutation or other mutations associated with GA biosynthesis or perception. Interestingly, previous research has linked breeding for the green revolution and dwarfing in rice genotypes has increased drought sensitivity in modern genotypes (Vikram *et al.*, 2015). Manipulating dwarf phenotypes using PGRs might be a strategy to improve drought tolerance in these genotypes.

Nevertheless, there was significant variation between genotypes in their root traits following treatment with GA3, with some genotypes having positive and some genotypes having negative responses (Figures 3.10-3.13 and 3.16-3.19) This could be explained through genetic variation in genotypes such as seed coat, which for some genotypes may allow more GA to pass through to the endosperm. However, there is still a lack of information about root growth regulation by gibberellins especially for rice. This is because little is known about transportation of GAs (Binenbaum *et al.*, 2018). Recent micrografting and biochemical analysis in Arabidopsis suggests that the GA precursor, GA12, is mobile in the xylem and phloem tissue and may allow long distance signalling of GA signals (Tanimoto, 2005, Regnault *et al.*, 2016).

These results are consistent with previous research using PGRs treatments in plants that has shown contrasting responses between shoot and root development (Overvoorde *et al.*, 2010, Gou *et al.*, 2010, Müller & Leyser, 2011, Shani *et al.*, 2013). Previous research, in which wheat seeds were soaked in the synthetic PGR, Uniconazole, which acts by inhibiting the production of gibberellins, increased water uptake and N accumulation in the subsequent seedlings, but it is not clear whether this was a consequence of reduced shoot growth or alterations to the root system (Han & Yang, 2009). However, soaking seeds in 10 µM GA had no effect on *Pisum sativum* root system growth (Tanimoto, 1987, 1988, 1994). In contrast, GA deficiency dramatically reduced root elongation in pea plants for isogenic lines of the *na* mutant compared with wild type. The *na* mutation is thought to inhibit GA12-aldehyde production from ent-7a-hydroxykaurenoic acid. Therefore, plants with *na* mutations were negatively affected root development (Yaxley *et al.*, 2001), suggesting the addition of GA may increase root elongation.

Auxin:

Previous research in Arabidopsis showed that growing plants in agar containing auxin reduced primary root growth (Kim *et al* 2001). Results from this study in which rice seeds were soaked in NAA, showed that NAA promoted primary root and total root growth in some genotypes and reduced them in others, highlighting significant genotypic variation for these responses (Table 3.1, Figure 3.23 -3.26). For lateral root count, auxin significantly ($P<0.001$) stimulated lateral root numbers in IR64, GHRAIBA and GHRAIBA 52 compared with their controls with no significant ($P>0.05$) difference in the other genotypes. Ruyter-Spira *et al.* (2011) found NAA and strigolactone (GR24) synergistically affected Arabidopsis plants, especially pre-treatment with NAA, which strongly stimulated the initiation of lateral root potential. This agreed with results for some genotypes (e.g. GHRAIBA), which suggested that soaking seeds in 250 µM NAA increased lateral root number (Figure 3.24).

Cytokinin:

The cytokinins had a significant negative effect on seedling growth. Seeds treated with Zeatin were significantly damaged as a result of the high concentration of NaOH required to dissolve the Zeatin. The negative effect of NaOH was confirmed when seeds of IR46 were soaked in 0.5 mM NaOH concentration (Appendix 1). Therefore, Zeatin was removed after first experiment with IR64. Since all cytokinins were initially dissolved in NaOH as the solvent (Table 2.2), soaking seeds in ADW as the control may not have been appropriate control, as residual NaOH may have altered the growth of the plants. Initial experiments with control treatments containing residual NaOH showed no effect on plant growth (data not shown), so in order to optimise the experimental design the additional control containing NaOH was not included. Investigating other NaOH concentrations may reveal the mechanism of the negative effects for this solvent.

For kinetin, there was no significant effects on root and shoot traits. The cytokinin, BA, had significant ($P<0.001$) negative effects on shoot and root traits. Rice seedlings, whose seed were soaked in BA showed decreased root and shoot traits (Figure 3.30- 3.34). These results agreed with Zaochang *et al.* (2000) where rice plants sprayed with 100 µM BA had reduced root and shoot traits compared to control plants. However, the results presented here disagree with (Liu *et al.*, 2011), where BA and nitrogen were applied to rice seedlings, stimulating tiller buds and worked synergistically to increase IAA concentrations.

While no previous studies have investigated pre-soaking of rice seeds with PGRs and the effect of such procedures on the later development of the plant, these results provide novel information about the rice root and shoot system, for the genotypes involved in this study after soaking in PGRs. Such information can provide better understanding for breeders about the root system of rice genotypes and to the potential of utilising PGRs to modify plant architecture. PCA analysis highlighted the important contribution of primary root length, lateral root counts and lateral root length in total root length. However, lateral root counts and lateral root length had a high significant contribution to total root length (Figure 3.9). Moreover, results in this study demonstrated that, variation in root architectures were significant between genotypes. Lateral root count and lateral root length had a considerable effect on total root length. This explains how genotypes NSICRC9, GHRAIBA, GHRAIBA 52 and IR64 swapped positions in the hierarchy of the twenty genotypes based on the lateral root count and lateral root length (Figure 3.23, Figure 3.24, Figure 3.25 and Figure 3.26).

Chapter 4. Impact of treating seeds of rice genotype GHRAIBA with 1000 µM GA3 on long term root development and drought tolerance.

4.1. Introduction:

Previous results in this thesis have demonstrated that treating rice seeds with GA stimulates both root and shoot growth, but the extent of this stimulation varies with genotype (Figures 3.11; 3.15; 3.18; 3.22). The hypothesis of using GA is to stimulate cell division and elongation for root system at early stage of plant growth which can help seedlings to establish strong root system has been demonstrated previously (Bari & Jones, 2009, Iqbal *et al.*, 2011, Verma *et al.*, 2016). This hypothesis for using GA is the halfway between the two mechanisms, stress avoidance and stress tolerance which are fully described in (chapter 1, section 1.9). GA hormone is well known for its role to regulate source-sink relationships and such regulation is important under stressed environments (Iqbal *et al.*, 2011, Albacete *et al.*, 2014, Roopendra *et al.*, 2018). In a study conducted on *Leymus chinensis* plants grown in pots and field conditions, seeds were soaked in 5, 10, 50, 100 and 200 µM GA3 (Ma *et al.*, 2018). Results showed that soaking seeds in 50 µM GA3 resulted in a significant (P<000.1) increase in germination, plant height, tiller number, and fresh and dry weight by 60%, 21%, 11%, 166% and 116%, respectively, compared with control (Ma *et al.*, 2018). Research in five wheat cultivars AARI-11, CHAKWAL-50, SHAHKAR, PAKISTAN-13 and FSD-08, where seeds were soaked in 100 µM GA3 were used to investigate wheat growth and development under drought conditions (Ulfat *et al.*, 2017). Results suggested seeds treated with 100 µM GA3 improved growth, development, yield and yield components under drought stress in the wheat plants (Ulfat *et al.*, 2017).

Based on experiments conducted in Chapter 3, where the effect of soaking seeds in different PGRs across twenty rice genotypes on root and shoot traits was investigated, the response of GHRAIBA to the 1000 µM GA3 in soil will be investigated further here. This will allow the effects of soaking seeds of this genotype in 1000 µM GA3 to be investigated beyond the seedling stage under drought conditions. GHRAIBA was selected based on the previous results which showed significant response from this genotype to the 1000 µM GA3 concentration up to the seedling stage (Figures 3.18; 3.21). Furthermore, GHRAIBA sub- species is Indica, which is most likely grow under arid and semi-arid environments worldwide. Therefore, a split plot experiment design was conducted to investigate the long-term effect of soaking seeds in PGR under well-watered and drought stress conditions in mature plants and to address the following hypotheses.

4.1.1. Aim and hypotheses

The aim of this chapter is to investigate the long-term effects of soaking seeds in PGR on rice root and shoot traits during development under well-watered and drought stress conditions.

Hypothesis 1. Soaking seeds of GHRAIBA with 1000 µM GA3 will increase seedling germination in soil.

Hypothesis 2. Soaking seeds of GHRAIBA with 1000 µM GA3 will increase root growth compared to plants whose seeds were soaked in water.

Hypothesis 3. Soaking seeds of GHRAIBA with 1000 µM GA3 will increase root growth under drought stress conditions compared to well-watered.

4.2. Material and methods:

4.2.1. Plant material and experimental design

Rice genotype GHRAIBA was selected to investigate the effect of soaking seeds for 24 hours in 1000 µM GA3 based on data presented in Chapter 3. In order to monitor root growth during plant growth a split plot design experiment was conducted inside a controlled-environment room using PVC plastic pipes (Yue *et al.*, 2006, Li *et al.*, 2017), Chapter 1, section 1.12). The main plot consisted of drought treatment or well-watered control, with the plot split with seeds soaked for 24 hours in 1000 µM GA3 or water (control) (Figure 4.1). Seeds of GHRAIBA genotype were soaked in 1000 µM GA3 or water as described previously (Chapter 2) and three seeds were planted in each pipe.

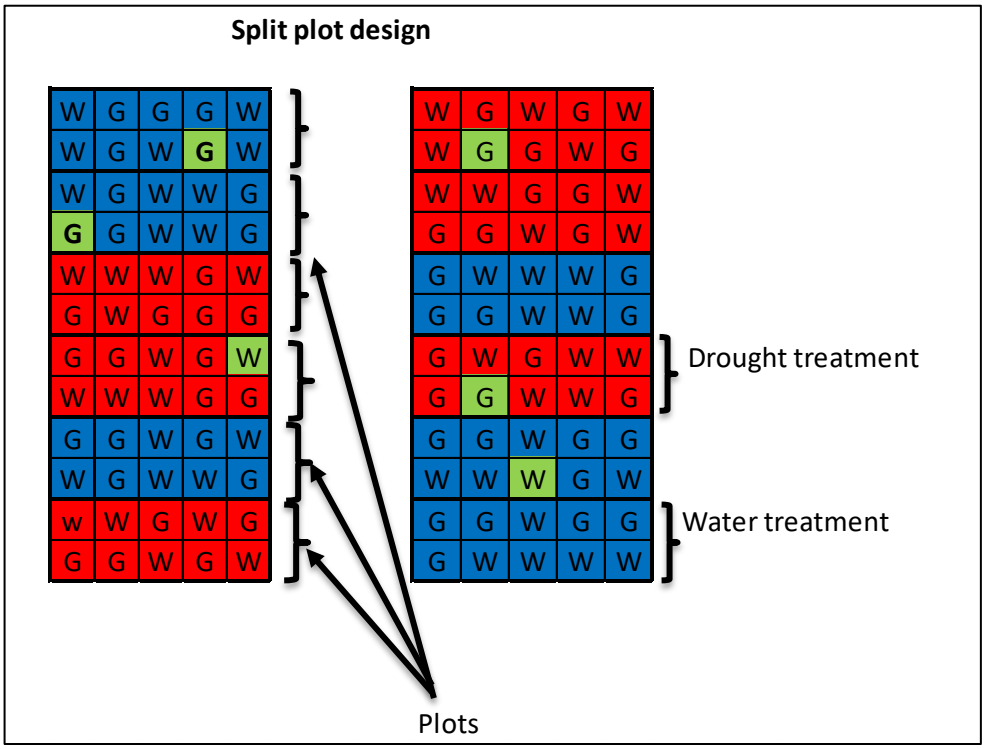


Figure 4.1. The experimental design for the experiment of treating seeds of rice genotype GHRAIBA with 1000 µM GA3 on long term root development and drought tolerance. Blue colour referred to well-watered treatment, red colour referred to drought treatment. W letter referred to the seeds soaked with water and G letter referred to the seeds soaked with 1000 µM GA3. Green colour explains the locations of the soil moisture sensors.

4.2.2. Plant growth system

PVC pipes (Wickes ltd. UK) 110 cm high and 10 cm in diameter were cut vertically into half and put back together using strong duct tape. Additional cable-ties were used to hold the two halves together. A plastic perforated cover was attached to the bottom for each pipe to maintain normal drainage (Figure 4.2).

Figure 4.2. Preparing pipes for root experiment. Pipes were cut vertically to two pieces and put back together using strong duct tape. Additional cable-ties were added to hold the two pieces of pipes together. A plastic perforated cover was attached to the bottom for each pipe to maintain normal drainage.

4.2.3. Growth substrate and conditions

Several initial experiments were conducted to specify the ideal soil-based substrate for growing rice and washing the roots after growing. Pipes were subsequently filled with topsoil/ lawn dressing (70% sand, 30% loam; Melcourt Industries Limited, Tetbury, Gloucestershire, UK). Osmocote 5-6 months was added to the soil as recommended 2-3 g/l (12g/ pipe) by (ICL Specialty Fertilizers, Ipswich, Suffolk, UK). The final substrate, including the Osmocote was analysed for available nutrients (NRM Laboratories, See Appendix 2).

Pipes were placed in walk in controlled growth room, with temperature set to 30 °C day and 26 °C night. The experimental conditions, more specifically day and night temperature for this experiment were different from chapter three to accommodate the whole plant growth period. Light and relative humidity were set to 12 h day and 60 %, respectively. The light intensity ranged between the 404-750 µmol PAR m^{-2} s^{-1}. light intensity inside the controlled environment was measured prior to start this experiment by using SKP 215 PAR quantum sensor to confirm the specified range. Day length was changed from 12 h to 10 h, when the plants finished the tilliering phase to prepare plants to reproductive stage. Rice knowledge bank calendar, for direct seeded rice were used as a guide development (Figure 4.3).

The light and temperature for this experiment conditions were selected based on the previous experiments as well as with a compromise with the conditions recommended by Cornell University (New York) for growing rice under a controlled environment (Harrington, 2010).

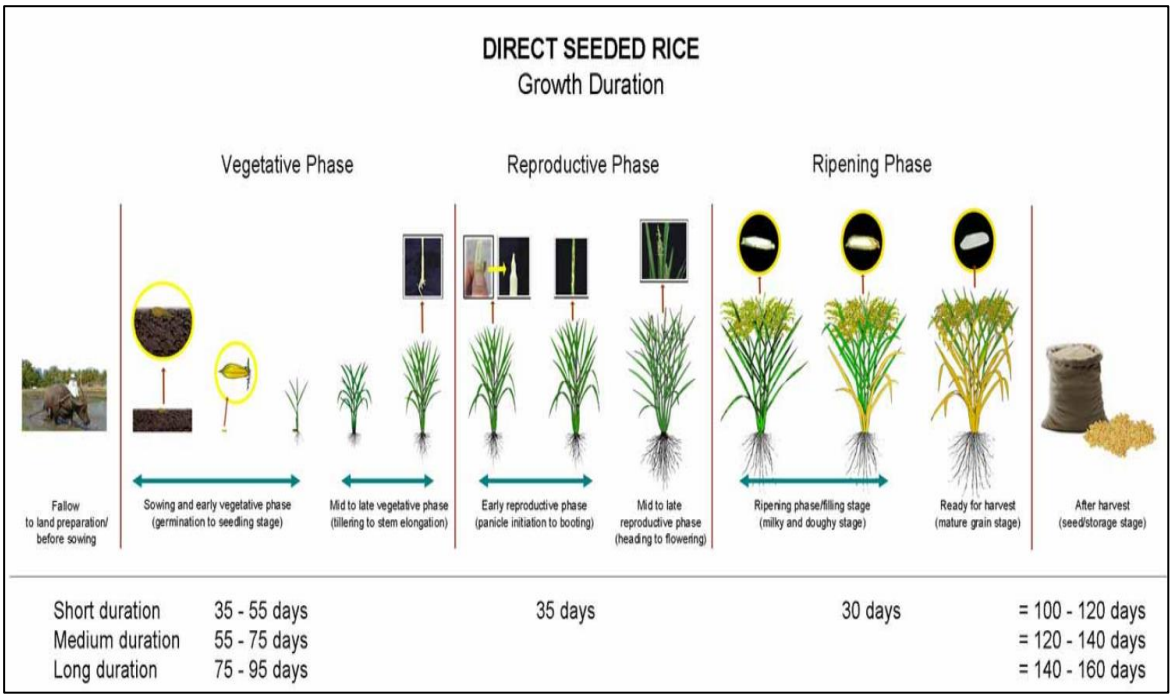


Figure 4.3. Growth duration diagrams presented as a crop calendar for rice seeds with direct sowing. Images from Rice knowledge bank.

4.2.4. Irrigation system

To create a drought stress treatment, an automated irrigation system using soil moisture probes to control drought treatments was designed (Figure 4.4). This system consists of four parts. 1) a Dosatron to deliver accurate concentrations of fertilisers to plants; 2) two solenoids valves controlling the water flow when triggered from the data controller; 3) The data controller used in this experiment was a GP2 (Delta-T Devices, Cambridge, UK); 4) soil moisture sensors, buried in the soil at the sides of the pipes to monitor the soil moisture level. The SM200 soil moisture sensors were used in this experiment with an error of ±3%, this sensor measures soil moisture content by creating a waveform signals through the steel rods which induce an electromagnetic field in the surrounding area of the sensor. This results in changing the permittivity of the water and the soil for the surrounding area and measuring the output voltage which reflecting the sensitive, simple measurement of soil moisture content. Sensor calibration was conducted for the soil prior to the start of the experiment. Test pipes were filled with the soil to be used in the experiments and 1.5L of water was added to each pipe and left for two days to measure the field capacity (Kirkham, 2014). Sensor readings were then check for field capacity using the SM200 soil moisture sensors. Based on preliminary experiments, sensors were placed at 15 cm from top of the soil and 60cm from top of the soil (Figure 4.5). These depths chosen because they represent the area where most change in soil moisture occurs. The GP2 data controller was programmed using the DeltaLINK 3.6 software.

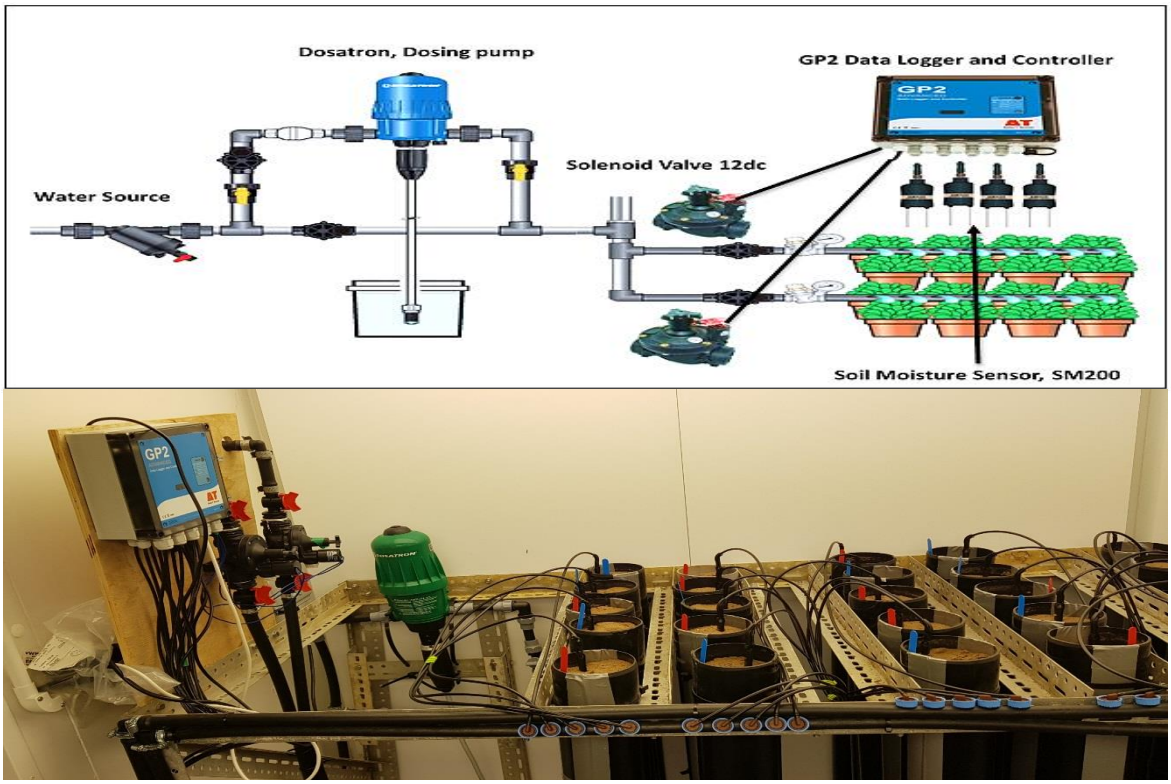


Figure 4.4. Irrigation system controlled using a GP2 data controller to control and monitor the irrigation process and a Dosatron (Water powered dosing technology) to control adding liquid fertilizers. GP2 controlled and monitored soil moisture levels by using SM200 sensors and triggering irrigation action to the two solenoid valves (12v dc), which control two different treatments, when the moisture level dropped below specified level for each treatment.

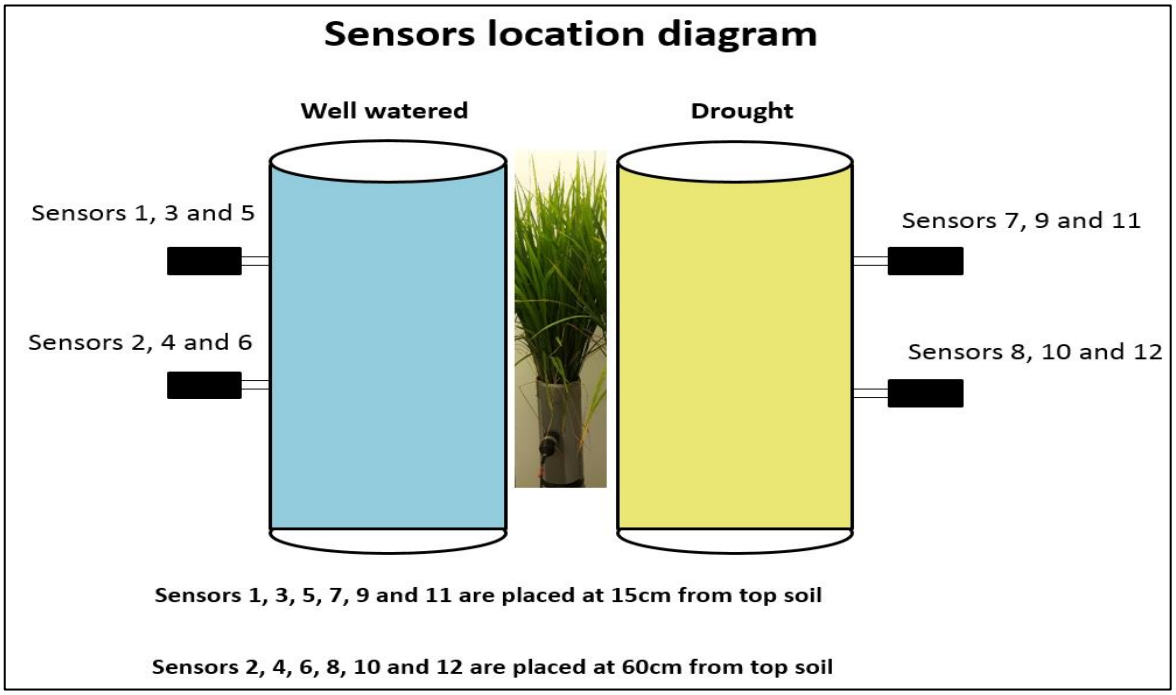


Figure 4.5. Soil moisture sensors locations and sensor numbers used to control irrigation for each treatment.

4.2.5. Activation and deactivation sensor values for the solenoid valves

The sensor values at which the solenoid valves would be activated and deactivated were specified using values from the soil moisture sensors placed at the top of the tubes. A value of 6 % for the soil moisture sensors represents 100% field capacity for the soil used in this experiment (70% sand and 30% loam). The average value for three soil moisture sensors located in the top of the pipes was used to trigger the irrigation action for well-watered and drought treatments (Figure 4.9).

Initially, 14 DAS in the well-watered treatment the solenoid valves were set to open when the average soil moisture value dropped below 3.5% and were set to close when the average soil moisture value was above 6%. In the drought treatment, the solenoid valves were set to open when the average soil moisture value dropped below 3 % and were set to close when the average soil moisture value was above 4.2 %. A value of 4.2 % was used to represent 70% of field capacity for the drought treatment based on preliminary trials (data not shown). Soil moisture sensor settings were subsequently adjusted based on soil moisture sensor readings and the development of the crop to maintain drought and well-watered conditions (Table 4.1).

Table 4.1. Soil moisture sensor settings used to control solenoid valves in the irrigation system used to impose well-watered and drought stress treatments. A value of 6% represents field capacity for the soil used.

Soil Moisture Sensor Settings				
Timepoint (DAS)	Well-watered treatment		Drought treatment	
	Solenoid Valve Open	Solenoid Valve Closed	Solenoid Valve Open	Solenoid Valve Closed
14	3.5%	6.0%	3.0%	4.2%
59	3.5%	7.0%	1.5%	2.8%
74	5.0%	9.0%	2.0%	4.0%
95	10.0%	15.0%	2.0%	4.0%
103	10.0%	15.0%	4.5%	6.0%

4.2.6. Plant growth and data collection

Seeds were sown on 25th January 2019. After sowing, all pipes were sprayed with tap water from the top, twice a day for 14 days before switching to automatic drip irrigation, to maintain adequate moisture level for seeds to germinate and gain good establishment. Seedlings were watered on 7th February with a full nutrient solution. A stock solution was prepared consisting of 1 kg of 1:1:1 Vitafeed balanced 19-19-19+1.6 MgO+TE in 10 L water. This was then further diluted to 1:100 using a diluter to fertigate the plants. On the 8th February, seedlings were watered again after they were thinned. Fertilisers were added based on the soil analysis (See appendix 2) and compatible with (Xu *et al.*, 2013).

4.2.7. Sample and crop measurements.

Germination percentage was recorded four, five and six days after sowing (DAS). Seedlings were initially thinned on 1st February 2019 to two seedlings per pipe (7 DAS). A second seedling thinning was conducted on 8th of February 2019 with seedling fresh and dry weight recorded (14 DAS). Plant height was recorded weekly up to three weeks after the second seedling thin.

Relative chlorophyll was measured 27 DAS using MultispeQ v2.0 device (PhotosynQ, East Lansing, USA). At the mid tiller stage (35 DAS), 60 tubes (half of the experiment) were harvested. Each tube was divided into four parts, with each one 25 cm in length (Figure 4.6) to investigate the distribution of roots down the soil profile. Soil was washed from the roots using a semi-automated root washing system (Figure 4.7; Clarke, 2017). The initial plan was to analyse the roots using WinRhizo programme at 300 dpi resolution (Expression 1600 XL-PRO, Epsom77 UK Ltd). However, due to the complexity of the acquired root system (Figure 4.8) and the limitation of the WinRhizo programme to measure the washed root, the fresh and dry weight was recorded.

For the final harvest total shoot fresh biomass, total dry biomass, number of tillers, panicle heads number, total yield/ plant and 100 seeds weight were recorded. Meanwhile, root fresh weight and root dry weight were measured as described previously. All dry weight samples were dried at 80 °C for 48 h.

Figure 4.6. GHRAIBA root architecture response to drought treatment and 1000 µM GA3 at the harvesting stage.

Figure 4.7. Root washer system. Water from the bottom reservoir is pumped into the bottom of the yellow buckets, creating a 'whirlpool' effect. Root-soil cores are place in the yellow buckets to separate the soil and root material. The lighter root material is then washers over a central overflow pipe and down into collecting funnels underneath the buckets, which contained mesh to collect the roots.

Figure 4.8. Root system collected from 25-50 cm section from plant whose seeds were treated with 1000 µM GA3 under well-watered conditions. Plants grow in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night.

4.3. Results

Following the activation of the drought stress, the mean of the soil moisture values for sensors located at the top of the well-watered tubes was 6-6.5%, until the end of the vegetative growth stage, when the setting was changed to meet the plant growth requirements (Figure 4.9). Whilst the values for the drought treatment were maintained to be 4% up to vegetative growth stage. There was a difference in soil moisture between the two treatments. Sensors placed at 60 cm were used to monitor the overall soil moisture (Figure 4.9). Soil moisture sensors readings were used to link irrigation with the plant phenology and the irrigation adjusted accordingly (Figure 4.10).

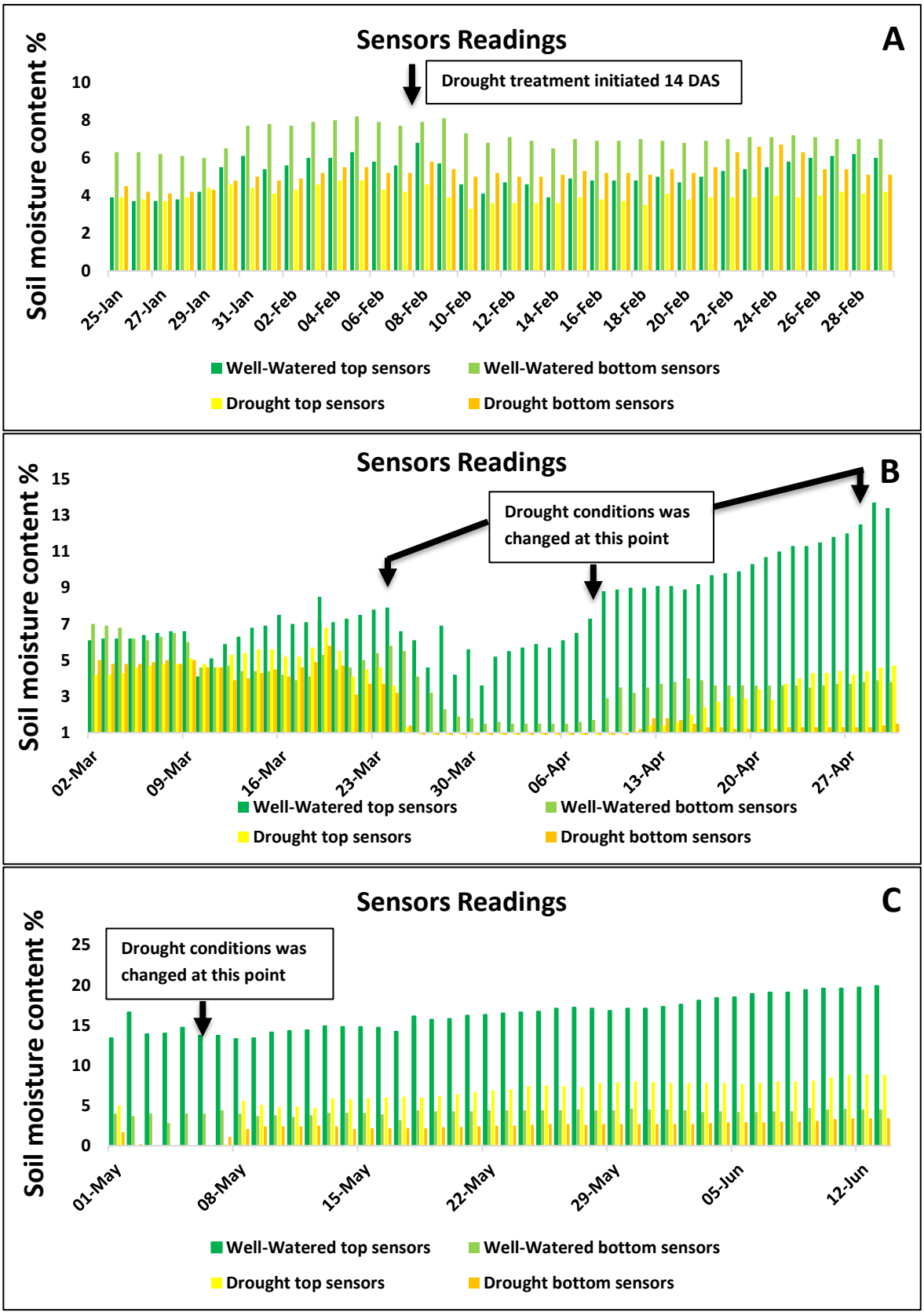


Figure 4.9. Soil moisture sensor data, for the whole experiment; A: Vegetative phase, B: Reproductive phase, C: Ripening phase.

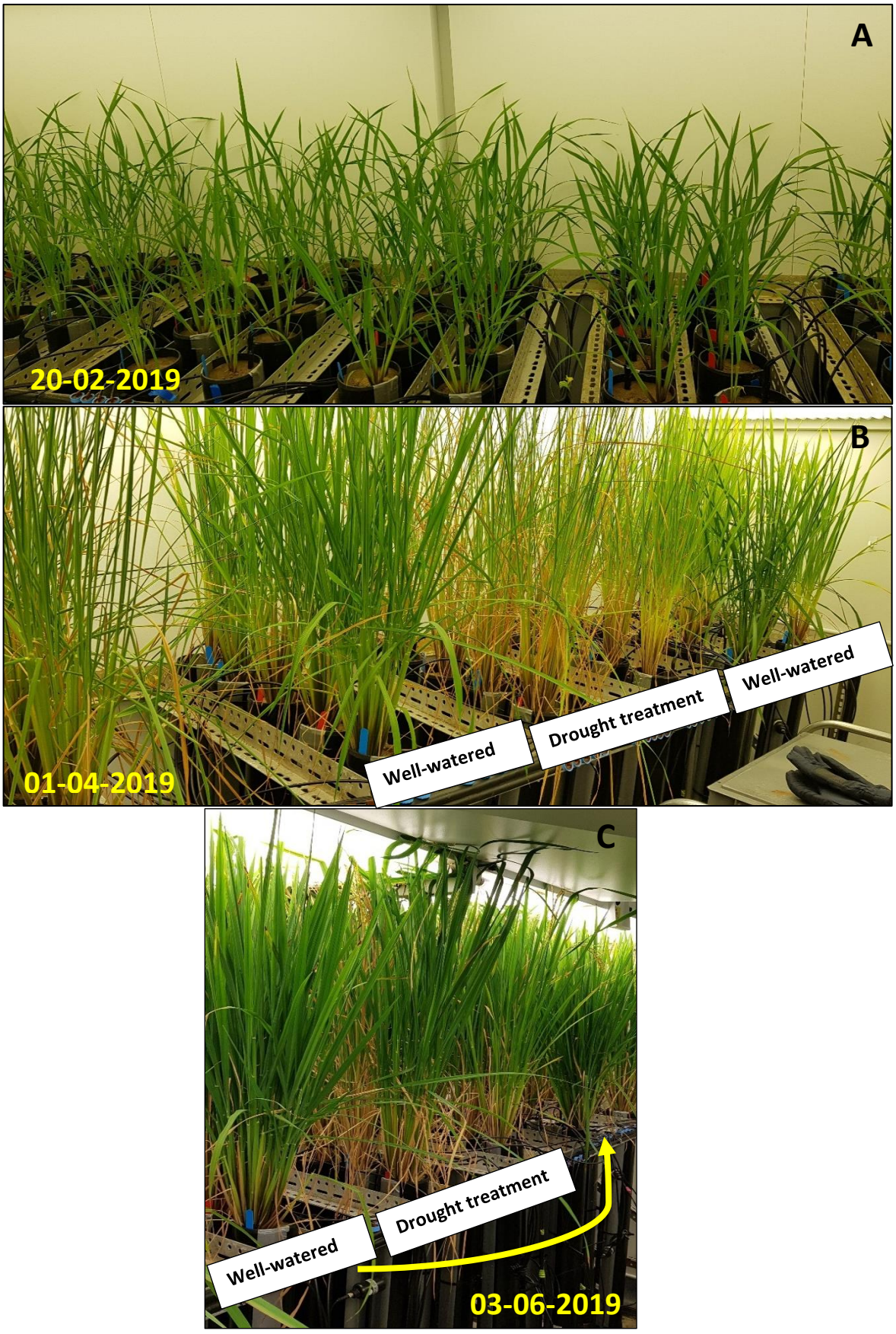


Figure 4.10. Different plant growth stages compatible with Sensors reading data. A: Vegetative phase, B: Reproductive phase, C: Ripening phase.

4.3.1. Effect of seed treatment on germination in soil.

Seed germination was scored 4, 5 and 6 days after sowing (DAS). Three seeds were planted in each tube. Four DAS, results showed a significant (P<0.001) difference in germination, with seeds soaked in 1000 µM GA3 for 24 hours having a significantly higher germination rate compared with seeds soaked in water (Figure 4.11). After 5 and 6 days after sowing there was no significant difference (P>0.001) between the two treatments (Figure 4.11).

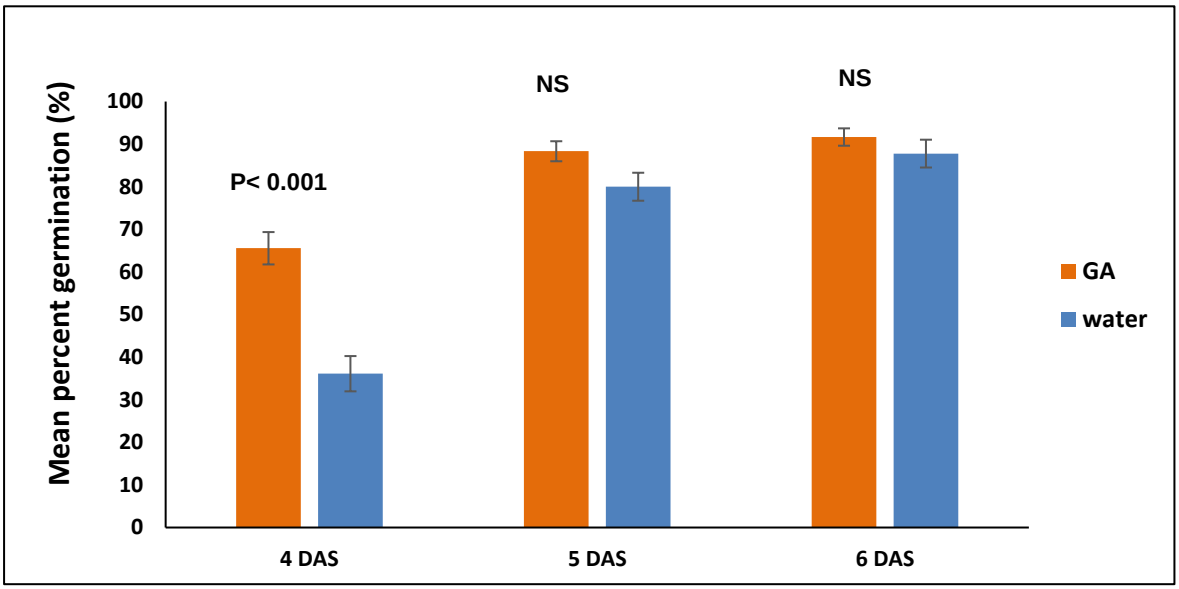


Figure 4.11. REML means for percentage germination 4, 5 and 6 days after sowing (DAS). Seeds were soaked for 24 hrs in 1000 µM GA3 or water before being sown into soil in a controlled-environment room set to 30/26°C day/night with 60% humidity and 12/12hrs day/night. Data shown are means ± SEM (n=60).

4.3.2. Seedling fresh weight seven and fourteen days after sowing.

Seven days after sowing (DAS) seedlings were thinned to two seedlings per tube, the fresh weight of the harvested seedlings was recorded. There was no significant difference (P>0.05) between seedlings fresh weight of the two treatments. Fourteen DAS, seedlings were thinned again to one plant per tube, the fresh and dry weight of the harvested seedlings were recorded. Results for seedling fresh weight, showed there was no significant (P>0.05) difference effect between the seeds soaked in 1000 µM GA3 and seeds soaked in water (Figure 4.12).

For seedling dry weight 14 DAS, there was a significant (P<0.05) difference between seedlings of seeds that had been treated with 1000 µM GA3 and control (Figure 4.13). The mean dry weight of seedlings whose seeds were treated with 1000 µM GA3 was 195.3 mg compared with control seedlings whose mean dry weight was 150.8 mg (Figure 4.13).

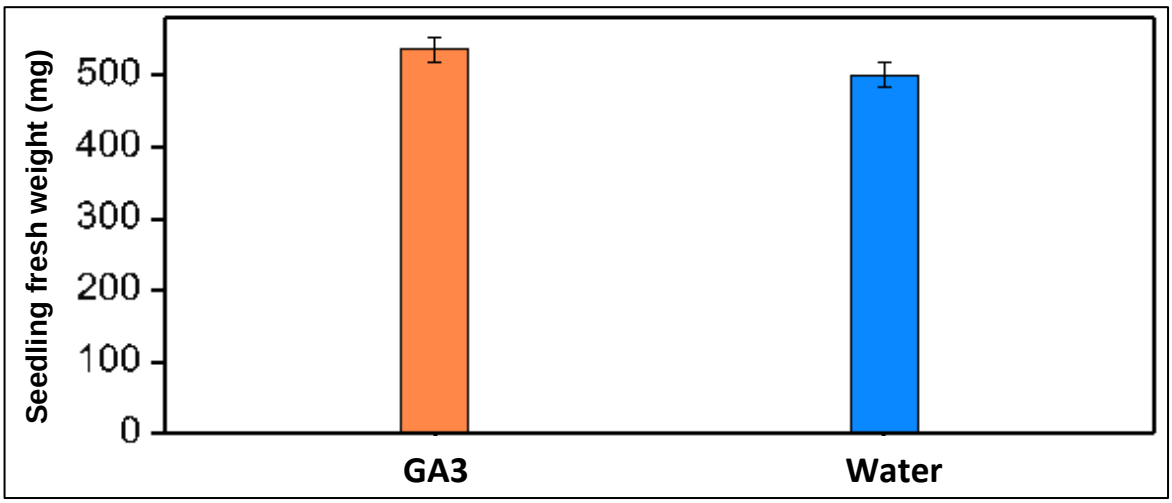


Figure 4.12. Mean seedling fresh weights for rice seedlings grown for 14 d. Seeds were soaked in either 1000 µM gibberellic acid (GA) or water for 24h prior to sowing. Seeds were sown into soil in 1m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100% field capacity (well-watered; blue bars) or 70% field capacity (drought stress; brown bars), although at this harvest point this treatment had not yet started. Seedlings were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data shown are means ± SEM (n=60).

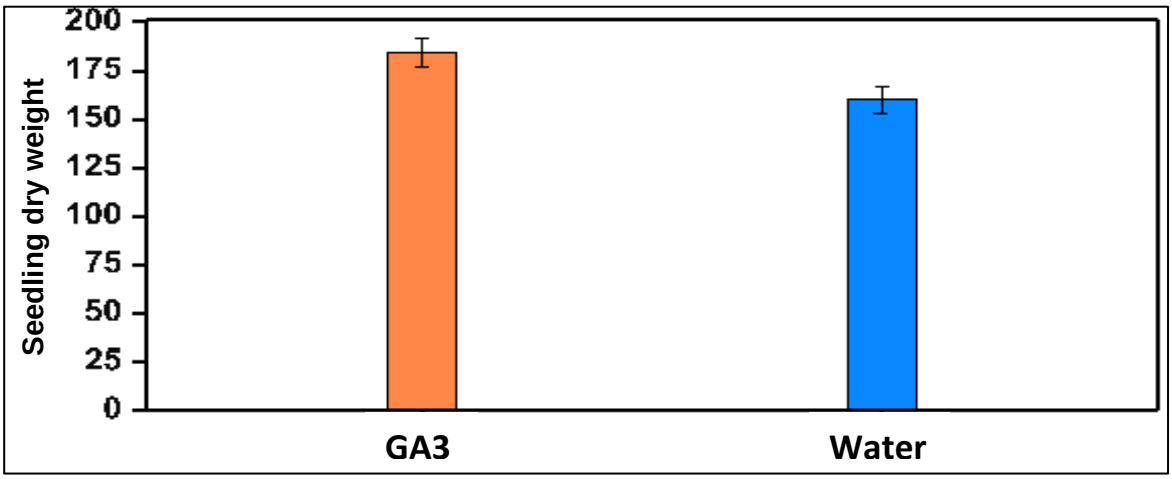


Figure 4.13. Mean seedling dry weights for rice seedlings grown for 14 d. Seeds were soaked in either 1000 µM gibberellic acid (GA) or water for 24h prior to sowing. Seeds were sown into 1m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100% field capacity (well-watered; blue bars) or 70% field capacity (drought stress; brown bars), although at this harvest point this treatment had not yet started. Seedlings were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. seedlings were harvested and dried in oven at 80 °C for two days, then measured. Data shown are means ± SEM (n=60).

4.3.3. Plant height.

Plant height was recorded weekly, 14 DAS for three weeks until it was no longer practical to measure the plant height in the growth room environment. The results showed, there was a significant (P<0.001) response, between plants whose seeds had been treated with 1000 µM GA3, which had a mean height of 33.03 cm and plants whose seeds were treated with water, which had a mean height of 31.18 cm. For plant height data collected 21 and 28 DAS plant height recorded was only significant (P<0.001) between drought and water treatment. Plant height under well-watered treatment was 42.6 cm and 53.6 cm compared with 46.9 cm and 59.9 cm for the drought treatment, for the second- and third week, respectively (Figure 4.14).

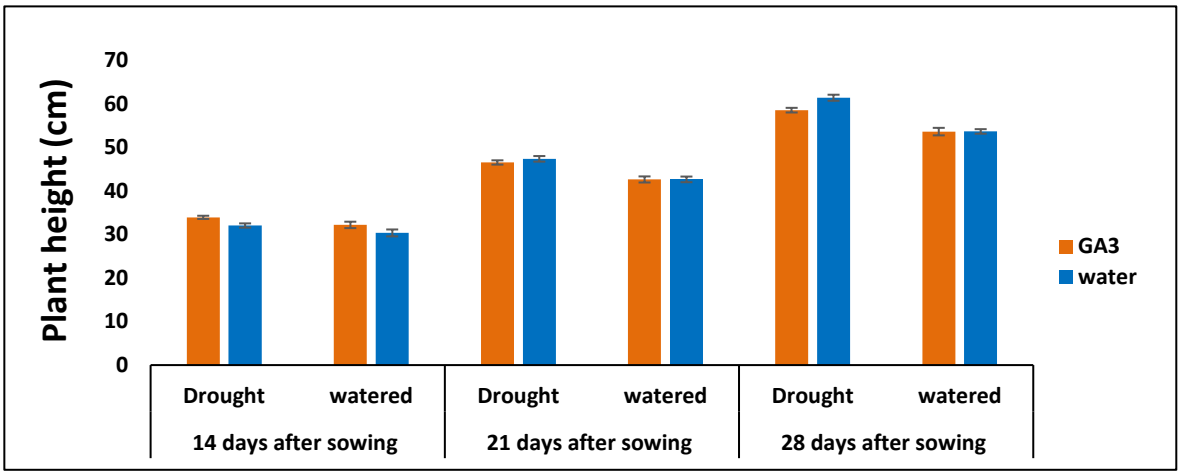


Figure 4.14. Mean rice plant height 14, 21 and 28 DAS. Seeds were soaked in either 1000 µM gibberellic acid (GA3) or water for 24 h prior to sowing. Seeds were sown into 1m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100% field capacity (well-watered; blue bars) or 70% field capacity (drought stress; brown bars). Seedlings were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data for 14 DAS was Log transformed for REML analysis to improve normality and distribution of variance and then back transformed for presentation in this figure. Data shown are means ± SEM (n=30).

4.3.4. Relative chlorophyll.

Twenty-seven DAS, relative chlorophyll content of leaves was recorded. The REML analysis for the relative chlorophyll contents showed a significant ($P<0.001$) effect of water and drought treatment, which had mean values of 38.3 and 43.5, respectively. There was no significant difference ($P>0.05$) between the seed treatments (Figure 4.15). However, the interaction between the drought stress and seed treatment was significant ($P<0.05$). Plants whose seeds were treated with 1000 µM GA3 and grown under drought stress had higher chlorophyll contents compared to those treated with 1000 µM GA3 under well-watered conditions (Figure 4.15).

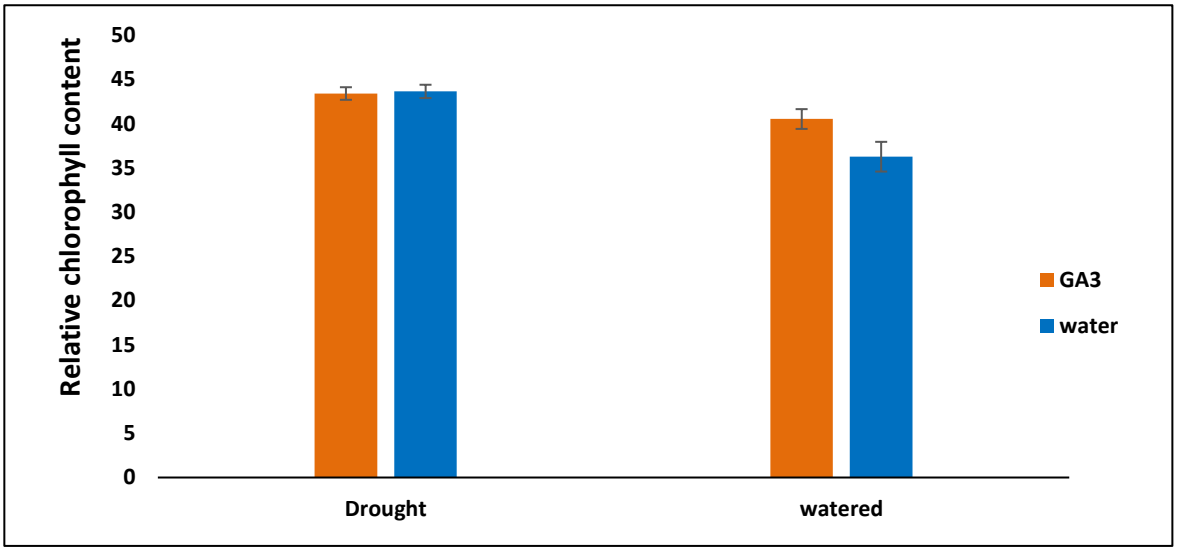


Figure 4.15. Mean relative chlorophyll content for rice plants grown for 27 d. Seeds were soaked in either 1000 µM gibberellic acid (GA3, brown bar) or water for 24 h (blue bar) prior to sowing. Seeds were sown into 1 m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100% field capacity (well-watered; blue bars) or 70% field capacity (drought stress; brown bars). Seedlings were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data shown are means ± SEM (n=30).

4.3.5. First stage (after 40 days) harvesting by the end of tillering stage.

By the end of tillering stage (40 DAS), half of the experiment (60 tubes) were harvested. This was to investigate and track the effect of treating seeds with 1000 µM GA3 on plant shoot and root growth for GHRAIBA. WinRhizo programme using a flatbed scanner (Expression 1600 XL-PRO, Epsom77 UK Ltd) was planned to measure root traits. However, due to large amounts of washed root acquired, fresh weight and dry weight were measured instead. Root systems were divided to four parts, with each part representing a 25 cm section of the tube (see section 4.2.7 and Figure 4.6). The results showed there were no significant ($P>0.05$) differences for the shoot fresh weight and tiller number between plants whose seeds had been treated with 1000 µM GA3 and those whose seeds had not been treated and between the drought and well-watered treatments and their interaction (Table 4.2). Meanwhile, there was no significant effect for root fresh and dry weight between the drought and well-watered treatments, as well as there was no significant effect between the 1000 µM GA3 and the water (Table 4.2)

Table 4.2. REML means of shoot fresh weight, tiller number, root fresh and dry weight 40 DAS. Seeds were sown into 1 m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100 % field capacity (well-watered) or 70% field capacity (drought stress). Seeds were soaked in either 1000 µM gibberellic acid (GA) or water for 24 h (Control) prior to sowing. Plants were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data shown for root fresh and dry weight were SQRT transformed for REML analysis to obtain normality and distribution of variance and then all transformed data was back transformed for presentation in this table. DAS refers to days after sowing. bars). Data shown are means ± SEM (n=15).

Water Treatment	**Seed Treatment**	**First Stage harvest 40 DAS**							
		Shoot Fresh Weight (g)		**Tiller Number**		**Root fresh Weight (g)**		**Root dry Weight (g)**	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Well-Watered	**GA**	51.87	4.34	11.87	0.59	37.6	0.13	10.1	0.08
	Control	50.33	4.51	12.67	0.87	42.8	0.15	12.9	0.09
Drought	**GA**	57.40	5.91	13.73	0.85	37.2	0.18	10.7	0.11
	Control	60.53	5.75	13.53	0.57	34.6	0.19	9.8	0.11
REML results	**Seeds treatment**	NS		NS		NS		NS	
	Drought Treatment	NS		NS		NS		NS	
	Interaction	NS		NS		NS		NS	

Nevertheless, analysis of the results showed there was high significant ($P<0.001$) differences between the divided root sections for the fresh and dry weight (Figure 4.16). The first two parts of root system contributed up to 70.9% of the whole root system for both fresh and dry weight (Figure 4.16).

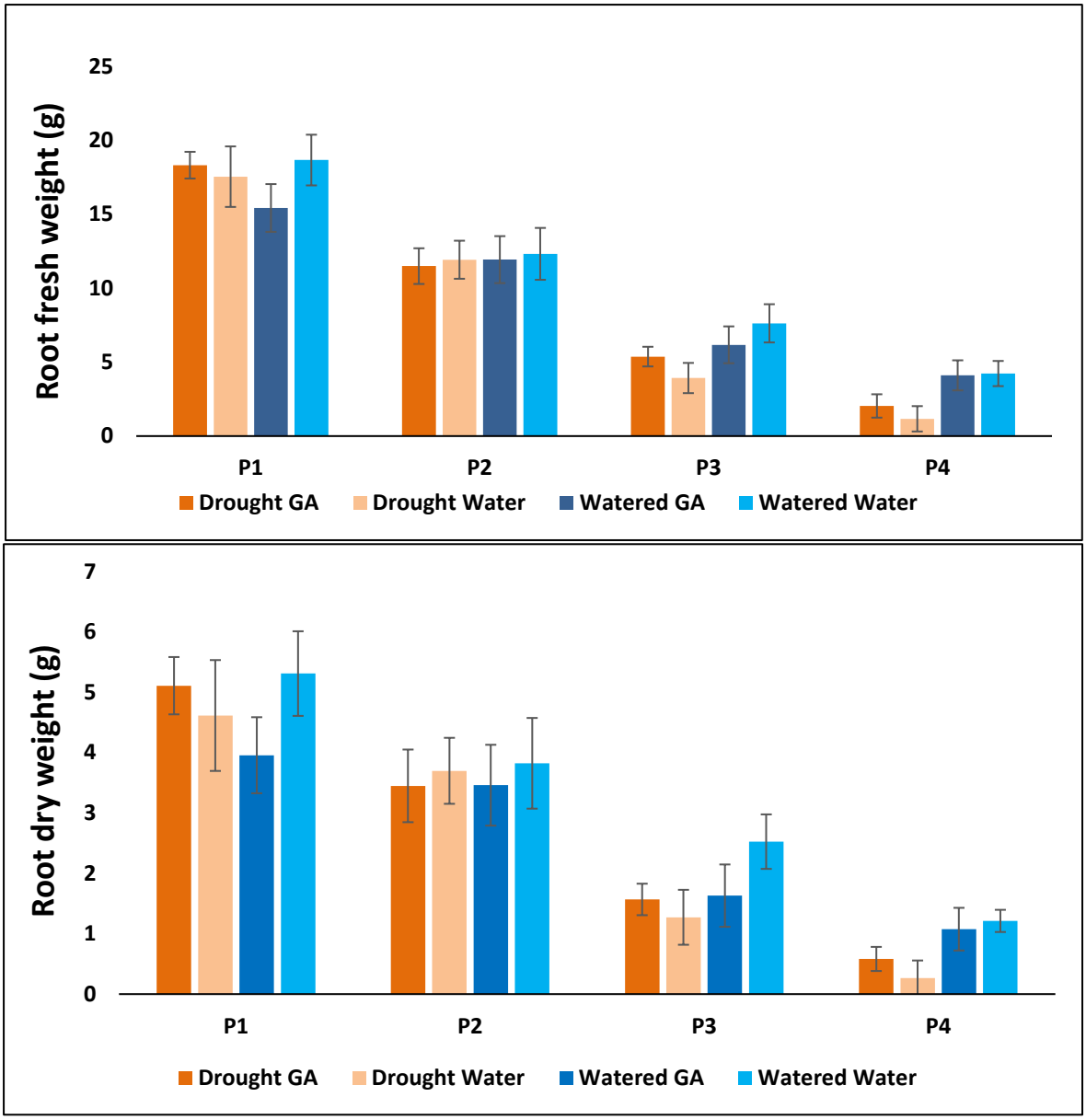


Figure 4.16. Mean fresh and dry weights of root systems parts (P1 = 0-25 cm, P2 = 25-50 cm, P3 =50-75, P4 75-100cm) 40 DAS. Seeds were soaked in either 1000 µM gibberellic acid (GA, dark brown or dark blue bars) or water for 24 h (Pale brown or pale blue bars) prior to sowing. Seeds were sown into 1 m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100% field capacity (well-watered; blue bars) or 70% field capacity (drought stress; brown bars). Plants were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data shown for root fresh and dry weight were SQRT transformed for REML analysis to obtain normality and distribution of variance and then all transformed data was back transform for presentation in this figure. Data shown are means ± SEM (n=15).

4.3.6. Final stage harvesting.

Analysis of the results for the final harvest at the end of maturity stage, showed that, there was no significant ($P>0.05$) difference between the water and drought treatments for the following traits, shoot dry weight, tiller number, panicle heads number and total yield plant (Table 4.3). However, there was a significant ($P<0.05$) difference between the well-watered and drought treatments for shoot fresh weight. There was also a significant ($P<0.05$) difference in 100 seed weight, between the well-watered and drought treatments and between plants whose seeds were treated with 1000 µM GA3 and those of plants whose seeds were not treated. Seeds collected from plants whose seeds were treated with 1000 µM GA3 had a mean 100 seed weight of 2.26 g under drought stress, compared with plants whose seeds were soaked in water and subjected to drought stress, which had mean 100 seed weight of 2.14 g (Table 4.3).

For root fresh and dry weight, there was no significant ($P>0.05$) differences between the well-watered and drought treatments or their interaction. However, there was a highly significant ($P<0.001$) difference between plants whose seeds were soaked in 1000 µM GA3 and those soaked in water for root fresh and dry weight (Table 4.4). Seeds treated with 1000 µM GA3 had 111.9 g and 122.9 g for mean root fresh weight under well-watered and drought treatment, respectively. Consequently, mean root dry weight was 38.2 g and 46.5 g for well-watered and drought treatment, respectively (Table 4.4).

Table 4.3. REML means of shoot fresh weight, dry weight, tiller number, panicle head number, seed yield and 100 seed weight at the maturity stage. Seeds were sown into 1 m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100 % field capacity (well-watered) or 70% field capacity (drought stress). Seeds were soaked in either 1000 µM gibberellic acid (GA) or water for 24 h (Control) prior to sowing. Plants were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data shown for root dry weight were SQRT transformed, for panicle heads number and seed yield data were Log transformed, for REML analysis to obtain normality and distribution of variance and then all transformed data was back transformed for presentation in this table. Data shown are means ± SEM (n=15).

Water Treatment	**Seed Treatment**	**Final stage harvest**											
		Shoot fresh weight (g)		**Shoot dry weight (g)**		**Tiller number**		**Panicle heads number**		**Seed yield (g plant^{-1})**		**100 seed weight (g)**	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Well-Watered	**GA**	109.80	3.44	83.17	0.188	30.80	1.19	13.15	0.044	10.84	0.059	2.33	0.04
	Control	107.3	2.87	79.46	0.140	27.47	1.26	13.12	0.043	10.52	0.060	2.31	0.05
Drought	**GA**	95.00	4.50	68.25	0.231	25.33	0.94	11.61	0.044	9.57	0.081	2.26	0.02
	Control	95.70	6.15	74.91	0.270	25.73	1.43	11.53	0.049	9.31	0.099	2.14	0.03
REML results	**Seeds treatment**	NS		NS		NS		NS		NS		P= 0.043	
	Drought treatment	P= 0.049		NS		NS		NS		NS		P= 0.020	
	Interaction	NS		NS		NS		NS		NS		NS	

Table 4.4. REML means for root fresh weight and dry weight at the maturity. Seeds were sown into 1 m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100% field capacity (well-watered) or 70% field capacity (drought stress). Seeds were soaked in either 1000 µM gibberellic acid (GA) or water for 24 h (Control) prior to sowing. Plants were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data shown for root fresh and dry weight were log transformed for REML analysis to obtain normality and distribution of variance and then all transformed data was back transform for presentation in this table. Data shown are means ± SEM (n=15).

Water Treatment	Seed Treatment	Final stage harvest			
		Root fresh weight (g)		Root dry weight (g)	
		Mean	SEM	Mean	SEM
Well-Watered	GA	111.9	0.03	38.2	0.03
	Control	100.1	0.02	35.2	0.03
Drought	GA	122.9	0.03	46.5	0.04
	Control	114.7	0.03	41.6	0.05
REML results	Seeds treatment	P=0.002		P=0.001	
	Drought treatment	NS		NS	
	Interaction	NS		NS	

For root system parts, the analysis showed there was highly significant (P<0.001) difference between the divided root sections for the root fresh and dry weight (Figure 4.17). The fact that first two parts of root system contributed up to 70.9% of the whole root system for both fresh and dry weight, was confirmed with the second stage harvesting (Figure 4.17).

Although, there was no significant differences (P>0.05) between the drought stress and well-watered on root fresh weight and dry weight, there was a significant difference (P<0.001) between plants whose seeds were soaked in 1000 µM GA3 and those soaked in water on mean root fresh and dry weight (Figure 4.17). Under

drought stress and for part four of the root system, plants whose seeds were soaked in 1000 µM GA3 had a mean root fresh weight of 15.7 g compared with 11.7 g for plants whose seeds were soaked in water. In addition, there was a significant difference (P<0.001) between drought and well-watered and their interaction with the root parts. Plants whose seeds were soaked in 1000 µM GA3 had mean root dry weight of 6.0 g under drought stress for part four of the root system, compared with 3.7 g for plants whose seeds were soaked in water (Figure 4.17).

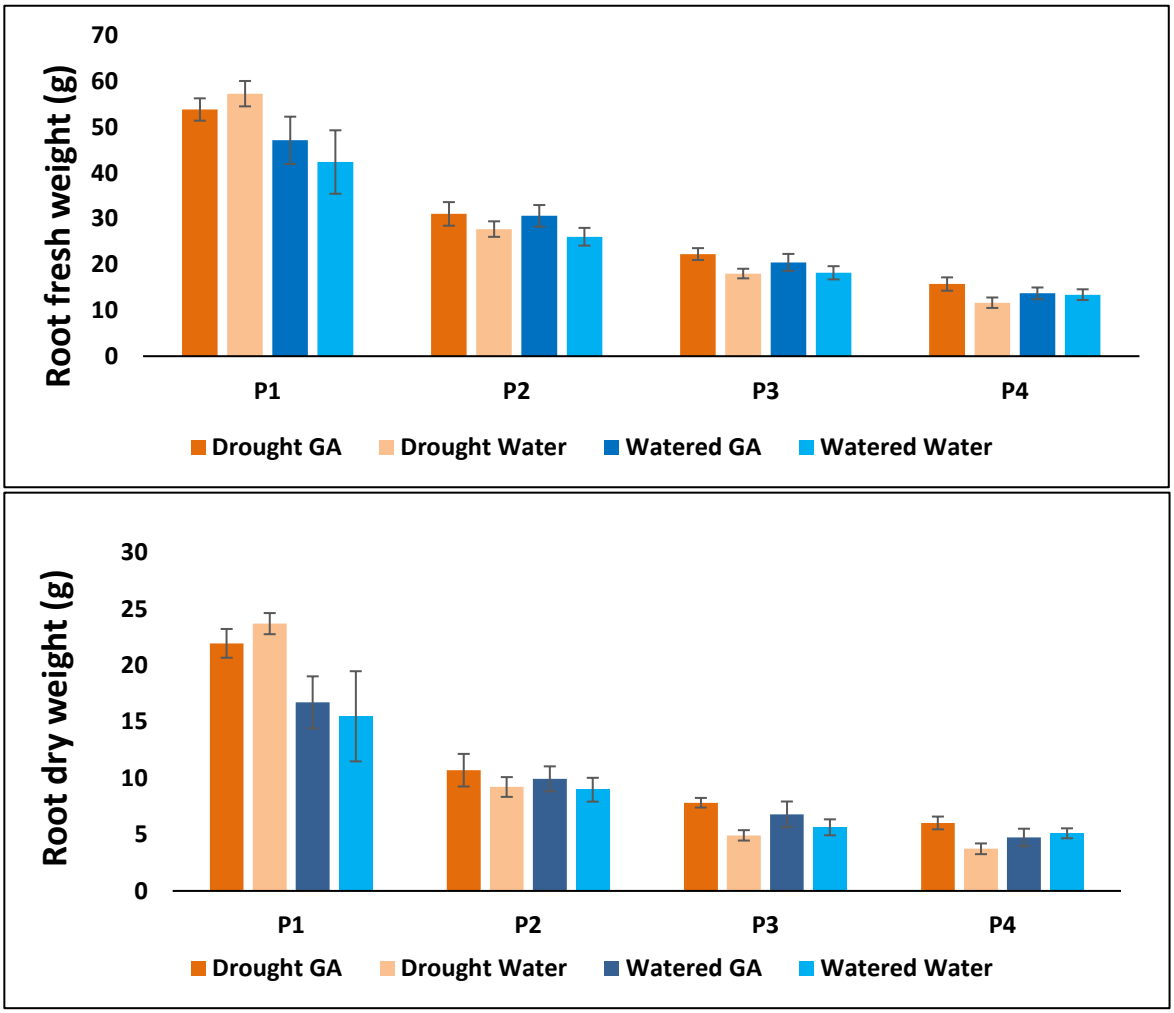


Figure 4.17. Mean fresh and dry weights of root systems parts (P1 = 0-25 cm, P2 = 25-50 cm, P3 =50-75, P4 75-100cm) at maturity. Seeds were soaked in either 1000 µM gibberellic acid (GA, dark brown or dark blue bars) or water for 24 h (Pale brown or pale blue bars) prior to sowing. Seeds were sown into 1 m tall drainpipes (dia. = 10 cm) that were irrigated to maintain 100% field capacity (well-watered; blue bars) or 70% field capacity (drought stress; brown bars). Plants were grown in a controlled environment set to 30/26°C day/night with 60% humidity and 12/12hrs day/night light. Data shown for root fresh and dry weight were LOG transformed for REML analysis to obtain normality and distribution of variance and then all transformed data was back transform for presentation in this figure. Data shown are means ± SEM (n=15).

Discussion

Previous results (Chapter 3) demonstrated that treating seeds with GA3 had a significant effect on root and shoot growth of seedlings. The aim of this Chapter was to determine if those effects continue into mature plants and whether they have potential benefits in tolerance to drought. Results confirmed the ability of the GA to increase germination 4 DAS compared with control treatment (Figure 4.11). However, there was no significant difference ($P>0.001$) between GA treatment and water treatment 5 and 6 DAS in the soil media (Figure 4.11). At 14 DAS plants whose seeds were soaked in 1000 µM GA3 had significantly ($P<0.05$) higher plant dry weights compared to control plants (Figure 4.13). This might be attributed to the benefit of stem elongation and accelerating the root establishment for the treated seedlings whose seeds were soaked in 1000 µM GA3. Furthermore, the results showed, there was a significant ($P<0.001$) response of plant height 14 DAS (Figure 4.14). These results agree with Ma *et al.* (2018) who suggested soaking *Leymus chinensis* seeds in 50 µM GA3 had a significant positive ($P<000.1$) effect on germination, plant height, tiller number, and fresh and dry weight.

For chlorophyll content, under the well-watered and drought treatment chlorophyll content was significantly ($P<0.001$) different. Plants under drought stress had higher chlorophyll content than the well-watered (Figure 4.15). Plant whose seeds treated with 1000 µM GA3 had significantly ($P<0.05$) higher chlorophyll contents than the plants whose seeds were treated with water under the well-watered treatment. This could be explained, through either, that drought stress reduced the water content of cells, or the low water potential might affect the chlorophyll concentration for the droughted plants. Thus, concentrating the chlorophyll per unit area. This result agreed with the results found by (Shah *et al.*, 2017) which demonstrated stress can increase total chlorophylls content. However, many papers report that drought

stress reduces leaf chlorophyll contents, which is contrast to the results reported here (Khadem *et al.*, 2010, Chen *et al.*, 2016). This may be attributed to several factors, including genotype grown, extent of drought stress or the method used to quantify chlorophyll content. Moreover, the results in this chapter imply that the impact of water stress at 70% of the field capacity on the studied plant traits may not have had a large effect on both biomass and root system development. For example, shoot biomass results show there was no significant (P>0.05) effect of the drought treatment. Consequently, it is difficult to confirm that the GA3 seed treatment had an effect in mitigating plant tolerance to drought under the current experimental design. Despite setting the irrigation system to provide 70% of the field capacity, imposing more water deficit, for example 60% or 50% of the field capacity with more replicates, may in future provide a greater drought response in the plants and answer the question to whether GA3 was able to modify the drought response.

At the first harvest 40 DAS, half of the experiment was harvested. The results of the harvested plants showed there were no significant (P>0.05) differences for the shoot and root system between plants whose seeds were treated with 1000 µM GA3 and those whose seeds were treated with water and between the drought and well-watered treatments and their interaction (Table 4.2). However, the results of this study showed that 70.9% of the root system was concentrated in the top 50 cm of the soil (Figure 4.16). At the final harvest at the maturity stage, the analysis of the results showed, there was no significant (P>0.05) difference between the water and drought treatments for the shoot dry weight, tiller number, panicle heads number and total yield plant (Table 4.3). Although, Kaur *et al.* (2015) concluded that soaking seeds in 100 ppm GA3 improved root length at later growth stages, results of this study only partially agree with this. During the early harvests (7 and 14 DAS) data showed a significant effect of soaking seeds in 1000 µM GA3 over the control.

However, the long-term effect of a single treatment of GA3 for seeds prior to sowing, was not sufficient to maintain the significant effect of the treated seeds seen at the seedling stage, over the mid and late plant growth stages. This was demonstrated with results obtained at the harvest 40 DAS (Table 4.2). Nevertheless, there was a significant ($P<0.05$) difference for 100 seed weight for plants whose seeds were treated with 1000 µM GA3 which had 2.26 g plant^{-1} compared to 2.14 g plant^{-1} for plants whose seeds were soaked in water under drought stress (Table 4.4).

Interestingly, when the root system was analysed in different sections the root fresh and dry weights in the lower part of the root system (P4) showed a significant difference ($P<0.001$) between plants whose seeds were soaked in 1000 µM GA3 compared to those soaked in water. Plants whose seeds were soaked in 1000 µM GA3 had a root fresh weight of 15.7 g compared with 11.7 g for control under the drought stress in P4 of the root system (Figure 4.17). Furthermore, GA treatment almost doubled the root dry weight in P4 for plants whose seeds were soaked in 1000 µM GA3 which was 6.0 g compared with 3.7 g for plants whose seeds were soaked in water (Figure 4.17). This may indicate that, although early response for GA was not significant (40 DAS), treatment with GA can give an advantage for root establishment at depth which may have benefits later in development. This result agreed with Sidiras & Karsioti (1996) who demonstrated that the main roots of lupins increased their length after the seeds were soaked in GA prior to sowing. This supports the hypothesis of using GA to have a significant effect on the early growth stage to establish strong root system, as GA stimulates cell division and elongation. Therefore, to further understand the role of GA3 in these changes to root systems, understanding the activation and deactivation of GA linked genes and their expression may have an advantage to achieve this objective. This aim will be investigating in the next chapter.

Chapter 5. Changes in gene expression of rice seedlings whose seeds were treated with hormones prior to germination

5.1. Introduction

Improving rice root system through understanding phenotype-gene regulation relationships is likely to result in increasing rice yield through breeding. This understanding can enhance the breeding for superior root traits which is considered crucial for improving tolerance to drought stress (Serraj *et al.*, 2011, Comas *et al.*, 2013, Nada *et al.*, 2019). Rice shoot growth is regulated and controlled by different PGRs in the same way as other plant's parts. The role of auxin and cytokinin in plant shoot growth have been investigated extensively (Scott, 1972, Werner *et al.*, 2001, Tanimoto, 2005, Werner & Schmülling, 2009, Peleg *et al.*, 2011, Lv *et al.*, 2018, Zha *et al.*, 2019). However, the interactions between plant phenotype, gene expression and PGRs still require further research to fully understand their individual roles and interactions (Wani *et al.*, 2016). This fact was highlighted when strigolactones (SLs) were used to investigate their control on tillering in rice. The results suggest that removing panicle significantly stimulate bud growth while bud growth was inhibited after GR24 application. Analysis of transcript expression in the buds uncovered that several genes involved in PGR signalling and treatment with GR24 supressed early auxin responsive genes in the bud (Wang *et al.*, 2018, Zha *et al.*, 2019). In another example, four rice genotypes Moroberekan, Giza178, PM12 and IR64 were investigated for expression pattern of root related genes (Nada *et al.*, 2019). Results suggested that, the expression of ARF12 and PIN1 auxin related genes may have a key role in improving root traits under drought stress. These genes increased the number of receptors on the surface of target root cells, which resulted in increased

root length in Moroberekan genotype under drought conditions and produced new and thicker roots in IR64 (Nada *et al.*, 2019).

Alternatively, a quantitative trait locus (QTL) approach may be the first step to identify the drought resistance responsible genetic loci. However, this may have some difficulties as drought is an interacting process between environmental factors, other abiotic stresses and plant development. Hence, rice genotype responses to drought in different mechanisms (See chapter 1, 1.8). Therefore, it might be difficult to allocate which rice genome regions are responsible for drought resistance under different drought stress environments. To overcome some difficulties associated with QTL , using genome-wide association studies maximizes the benefit of QTL approach which can be used to highlight some drought resistance-related genes (Price *et al.*, 2002). Activating or deactivating genes associated with QTL could help to fill the gap between genes which are responsible for morphological and physiological traits and their functions for rice root growth and development under drought stress. Genes associated with drought responses identified through QTL and/or genome wide association studies can vary between crops and even within the same crop under different environments or with different populations of genotypes. Therefore, studying QTL of root system traits for one group of rice genotypes may not reflect the full understanding of other genotypes root systems (Uga *et al.*, 2013, Uga *et al.*, 2015, Meng *et al.*, 2019).

However, quantitative traits like root length are more likely to be controlled by more than a single gene (Table 5.1). In response to drought stress, cloning and characterization of DEEPER ROOTING 1 (*DRO1*) gene was studied by (Uga *et al.*, 2013). The results suggested that, DRO1 was responsible for increasing the root growth angle. Backcrossing the Kinandang Patong genotype which carried this gene with IR64, which is classified as a shallow-rooting genotype, root length was

improved for IR64 genotype and enhanced the drought avoidance mechanism. Interestingly, auxin negatively affected the DRO1 gene expression (Uga *et al.*, 2013). Using all available genetic sequences for rice at the time, Sakamoto *et al.* (2004) identified all genes encoding proteins involved in the GA biosynthetic pathway. Twenty-nine candidate genes were identified across nine of the 12 rice chromosomes. The identified genes were *OsCPS1-4*, *OsKS1-9*, *OsKO1-5*, *OsGA20ox1-4*, *OsGA3ox1 and 2*, *OsGA2ox1-4* and *OsKAO*. Among these genes, *OsCPS1*, *OsCPS1-1* and *OsGA2ox1* were highly expressed in root tissues and may be involved in root growth and development. In contrast, the mutant *osko2-1* had negative effect on growth overall (Sakamoto *et al.*, 2004). The study demonstrated that, single gene is more likely to be responsible for coding the enzymes during the early stages of GA biosynthetic. While, during the later GA biosynthetic enzymes are more likely to be encoded by gene families.

5.1.1. Aim and hypothesis

The aim of this chapter is to assess the effect of soaking of GHRAIBA seed with 1000 µM GA3 on gene regulation and the expression of genes related to drought stress.

Hypothesis 1. Soaking seeds of GHRAIBA with 1000 µM GA3 will upregulate growth related genes compared to plants whose seeds were soaked in water.

Table 5.1. Genes name and suggested function in the recent studies about rice stress tolerance.

QTL or Gene name	Known as	Function	Reference
DEEP ROOTING 1	*DRO1*	A quantitative trait locus controlling root curvature gravitropic.	(Uga *et al.*, 2013)
AUXIN RESPONSE FACTOR12	*ARF12*	Responsible for regulating root elongation and support auxin transportation by activating transcription factors.	(Nada *et al.*, 2019)
PIN FORMED	*PIN1*	Stimulate adventitious root and tillering evolution.	(Xu *et al.*, 2005, Nada *et al.*, 2019)
Abiotic Stress Responsive	*OsASR1* *OsASR3*	Inducing drought tolerance in transgenic rice	(Joo *et al.*, 2013)
Dehydration-responsive element-binding	*OsDREB1A* *OsDREB1B*	Improve drought tolerance, high-salt and low-temperature stresses by encoding proteins which are responsible for drought tolerance such as ethylene-responsive factor (ERF)/APETALA2 (AP2)-type and binding proteins (DREBs).	(Ito *et al.*, 2006)
NAC transcription factor	*OsNAC6*	Work as transcriptional activator for drought, high salinity, wounding and blast disease.	(Nakashima *et al.*, 2007)
AT-hook content nuclear localized protein	*OsAHL1*	This gene activated and deactivated was linked with plant hormones such as ABA, H2O2, JA and SA.	(Zhou *et al.*, 2016)
Plasma membrane-type	*OsPIP2;4* *OsPIP2;5*	Expressed a controlling of rice aquaporins and high-water channel activity when water influx or efflux	(Sakurai *et al.*, 2005)
GA2ox genes	*OsGA2ox1*	Most likely expressed in root system, mature and immature panicles.	(Sakamoto *et al.*, 2004)

5.2. Material and methods.

5.2.1. Plant material and growth

Seeds of GHRAIBA genotype were soaked in 1000 µM GA3 or water (control) for 24 h as described in Chapter 2. After soaking, seeds were placed on moist filter paper and placed in an incubator for 7 d as described in Chapter 2.

5.2.2. Total RNA extraction

Total RNA was extracted from root and shoot samples separately, after the seedling growth for 7 d (Chapter 2) using the Spectrum Plant Total RNA kit (Sigma-Aldrich Company Ltd. Gillingham, Dorset, UK, Catalog Nos. STRN250) according to the manufacturer's instructions. On-Column DNase Digestion was used to complete removal of traces of DNA during RNA purification (Sigma-Aldrich Company Ltd, Catalog Nos. DNASE70).

5.2.3. RNA quality control

The quantity of the harvested RNA was checked by using two devices, NanoDrop 2000 Spectrophotometer (Table 5.2; Thermo Fisher Scientific, Wilmington, DE 19810 U.S.A.) and Qubit® 3.0 Fluorometer (Table 5.3; Life Technologies Holdings Pte Ltd) following manufacturer's instructions for each device.

Table 5.2. RNA samples checked by using NanoDrop-2000 device.

Samples	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
GA Shoot	92.4	ng/µl	2.30	1.12	2.06	2.15
Water Shoot	58.7	ng/µl	1.47	0.71	2.05	2.07
GA Shoot	99.6	ng/µl	2.48	1.19	2.08	2.09
Water Shoot	885.5	ng/µl	22.13	10.47	2.11	2.23
GA Shoot	506.6	ng/µl	12.60	6.17	2.05	2.26
Water Shoot	669.5	ng/µl	16.70	7.85	2.13	2.41

Table 5.3. RNA samples checked by using Qubit device.

Samples	Assay Name	Qubit® tube conc.	Units	Original sample conc.	Units	Sample Volume (µL)	Dilution Factor
GA Shoot	RNA High sensitivity	1000	ng/mL	40	ng/µL	5	40
Water Shoot	RNA High sensitivity	660	ng/mL	26.4	ng/µL	5	40
GA Shoot	RNA High sensitivity	780	ng/mL	31.2	ng/µL	5	40
Water Shoot	RNA High sensitivity	510	ng/mL	20.4	ng/µL	5	40
GA Shoot	RNA High sensitivity	428	ng/mL	17.1	ng/µL	5	40
Water Shoot	RNA High sensitivity	435	ng/mL	17.4	ng/µL	5	40

All samples had A260/280 and A260/230 rations greater than 2, suggesting they were free from contaminants (Table 5.2). Total RNA concentrations of samples sent for sequencing were diluted and checked using the Qubit in line with the sequencing requirements (Table 5.3).

At this stage insufficient RNA was extracted from the root samples, so these were not used for sequencing. To confirm the integrity of the RNA and confirm no degradation had taken place, RNA samples were run on denaturing formaldehyde gel in MOPS buffer (Figure 5.1).

RNA Loading Buffer (1X MOPS) was made on the same day and all samples and ladder were heated at 70 °C for 10 min prior to loading. Samples were run on the gel at 60V for 90 minutes.

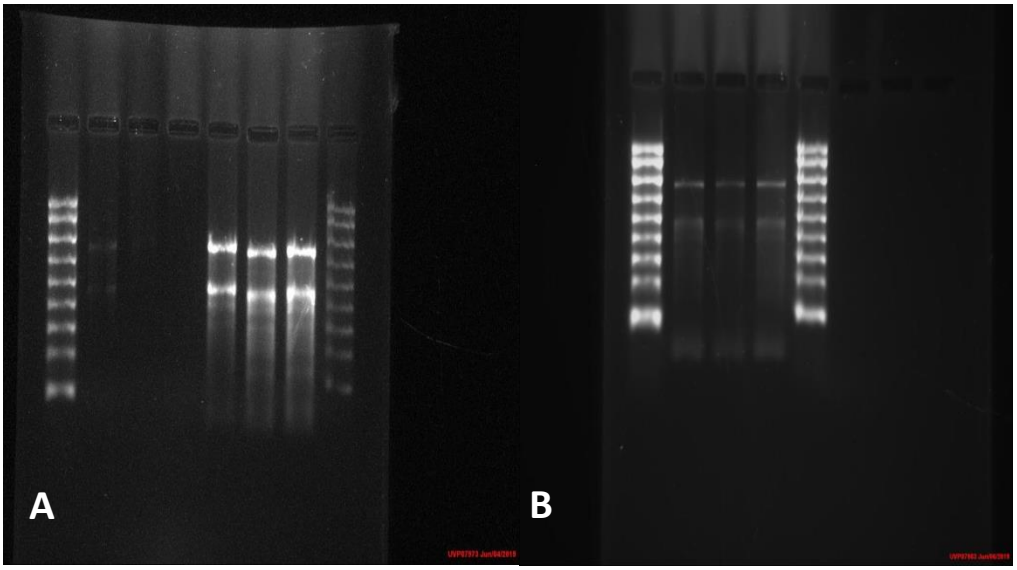


Figure 5.1. Gel images for RNA (A) samples extracted from shoots of seedlings whose seeds were soaked in GA3 and (B) samples extracted from shoots of seedlings whose seeds were soaked in water, to confirm there was no degradation of the RNA. Samples were run on denaturing formaldehyde gel at 60V for 90 minutes. Extracted RNA from the root samples was insufficient at this stage.

5.2.4. RNA sequencing

Total RNA samples were then sent for sequencing on an Illumina NovaSeq 6000 using a 150bp paired end read at the Oxford Genomics Centre (Oxford Genomics Centre, Oxford, UK).

5.2.5. Data analysis

Raw sequence data were checked and trimmed by Oxford Genomics Centre and sequences were aligned to the reference genome for Oryza indica ASM465v1 (https://plants.ensembl.org/Oryza_indica/Info/Index) using the Oxford Genomics Centre alignment pipeline. Data were further analysed using the SeqMonk software package(version 1.45.4; https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/) to quantify transcript counts and determine significantly differentially expressed transcripts. Raw data were checked for duplication and for consistency between treatments and replicates using the Duplication Plot and Data Store Similarity respectively. Data were processed to provide raw counts per gene and quantified using DESeq2 to identify differentially regulated genes that had a corrected P value of <0.05.

5.3. Results

5.3.1. RNA sequencing quality

After soaking the seeds of GHRAIBA genotype in 1000 µM GA3 or water for 24 h, seeds were placed on moist filter paper and placed in an incubator for 7 d. RNA was extracted from root and shoot samples separately. The quantity of the harvested RNA was checked by using NanoDrop 2000 Spectrophotometer and Qubit® 3.0 Fluorometer (see section 5.2.3). However, the extracted RNA from the root samples were insufficient, so these were not used for sequencing. Consequently, RNA samples extracted from shoots of seedlings whose seeds were soaked in GA3 and water were checked on denaturing formaldehyde gel in MOPS buffer. Initial library preparation for sequencing failed. The libraries were prepared again using fresh RNA, but the resulting libraries were not ideal. Due to time and cost restrictions, it was not possible to produce more RNA samples for sequencing. Consequently, the libraries were sent for sequencing.

Sequencing was carried out using Illumina NovaSeq 6000 (Table 5.4). An average of 136 million reads were sequenced per sample giving an average total read length per sample of 14.8 Gbp per sample. This represented an average of 38-fold coverage across the rice genome. The sequences were aligned to the *O. sativa* indica reference genome with an average read alignment of 85 % across all samples (Table 5.4).

Table 5.4. Sequence metrics for RNA samples extracted from rice shoots seedlings whose seeds had been treated with GA or water prior to germination.

Sample	Total read count (millions of reads)	Mean read length	Total read length (Gbp)	Fold coverage (%)	Read alignment (%)
GA treated seeds Rep 1	118.7	115	13.73	35.9	80.56
GA treated seeds Rep 2	132.7	111	14.73	38.5	87.29
GA treated seeds Rep 3	143.7	107	15.40	40.2	87.76
Water treated seeds Rep 1	114.1	113	12.95	33.8	83.63
Water treated seeds Rep 2	153.2	102	15.70	41.0	87.76
Water treated seeds Rep 3	154.5	106	16.40	42.8	87.97

5.3.2. Data processing

Given the issues with library preparation, samples were analysed for duplication levels (Figure 5.2). All samples showed a high level of duplication, with replicate one of both GA and water treated seeds having the highest levels of duplication. It is not clear what caused the duplication, which may be attributed to library preparation or the natural presence of a few dominate transcripts. As Sayols *et al.* (2016) refers to, in RNA-Seq natural over-sequencing of highly expressed genes may result in duplication, which are not easy to discriminate from normal read duplication. This high duplication rates could result from problems in different steps of library preparation process. However, all shoot samples met quality control thresholds before being sent for sequencing and prior to library preparation (Tables 5.2 and 5.3). Consequently, the high levels of duplication observed here are not ideal, some of this duplication may be the consequence of a few highly expressed transcripts.

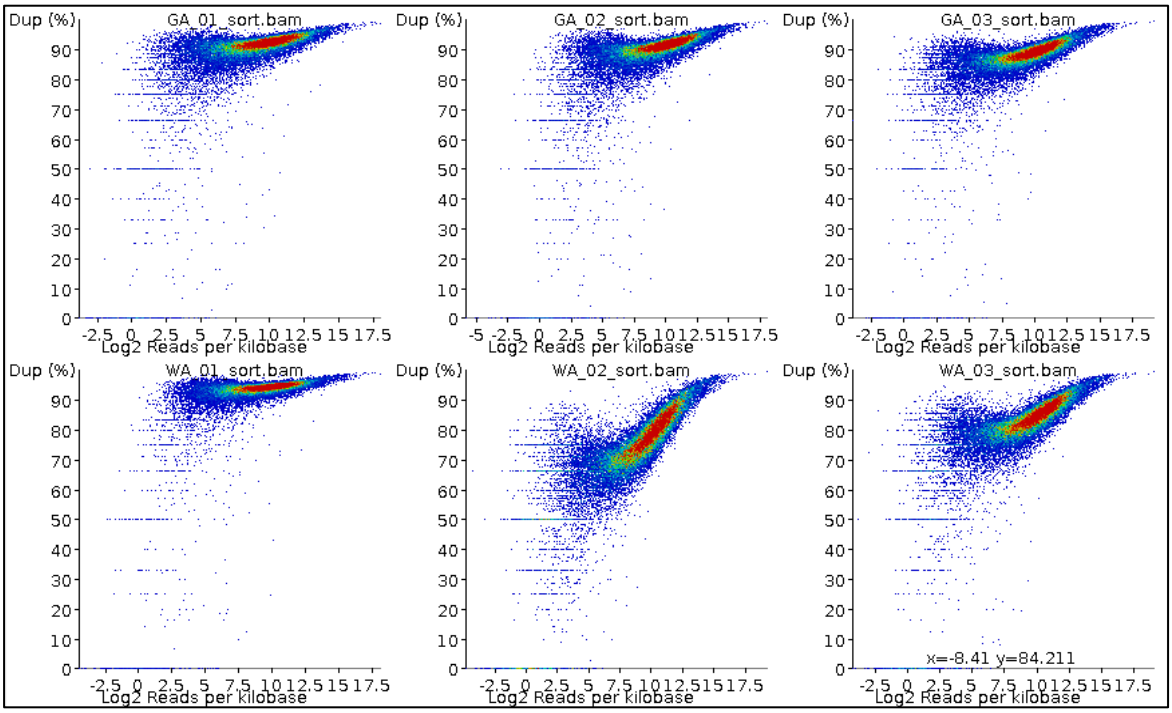


Figure 5.2. Duplication plots for RNAseq samples generated using the Duplication plot function in SeqMonk analysis package. Samples are from 7 d old seedlings whose seeds were treated with 1000 µM GA3 (GA) or water (WA) prior to germination. Three independent replicates were analysed (denoted by 01, 02, 03) for each treatment. Sequences were generated using Illumina NovaSeq 6000 with 150 b Paired end read. Y axis refers to the duplication level, while X axis refers to log2 reads/ kilobase. Colours in the scatterplot represent the density of points which are overlaid at that point in the plot, moving from blue (single data point) to red (multiple data points).

Samples were also clustered to check for similarities between treatments using PCA (Figure 5.3). The first two PCAs accounted for 75% of the variation in the data set, but there was no clear grouping of the samples, by treatment or replicate.

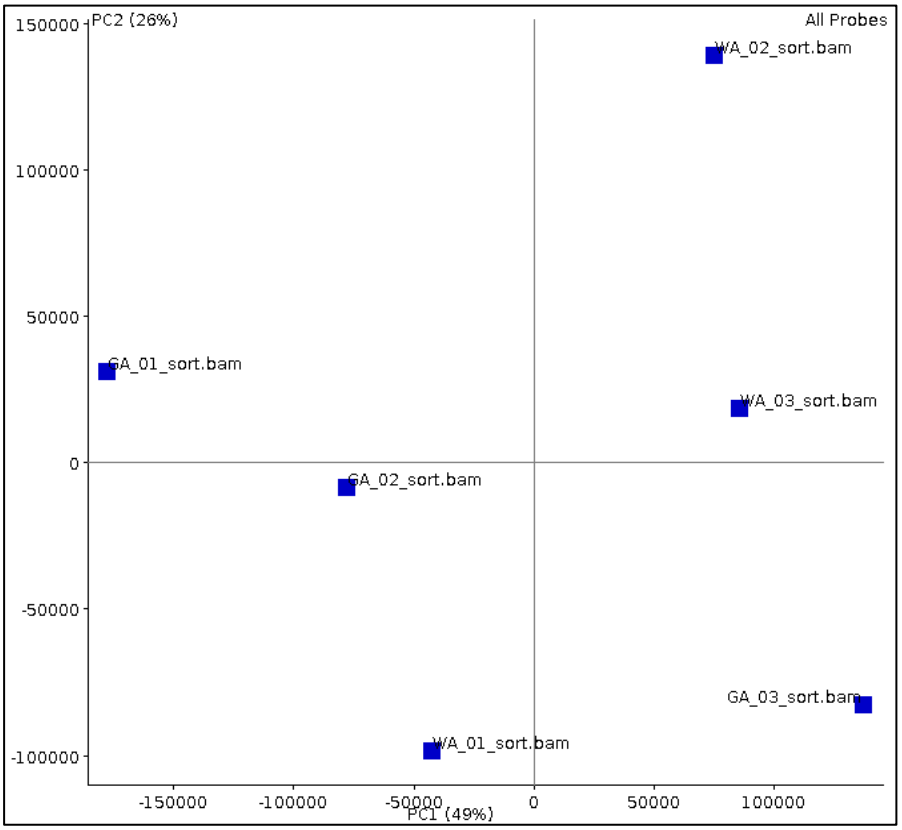


Figure 5.3. Principal component analysis of RNAseq samples generated using the Data Store Similarity function in the SeqMonk analysis package. Samples are from 7 d old seedlings whose seeds were treated with 1000 µM GA3 (GA) or water (WA) prior to germination. Three independent replicates were analysed (denoted by 01, 02, 03) for each treatment. Sequences were generated using Illumina NovaSeq 6000 with 150 b Paired end read.

5.3.3. Differentially expressed genes

Only six genes were identified as being differentially expressed between seedlings whose seeds were soaked in 1000 µM GA and those soaked in water prior to germination. One gene was upregulated, and five genes were downregulated (Table 5.5). Given the limited set of differentially expressed genes further analysis was limited.

Table 5.5. Upregulated and downregulated genes resulted from RNA Sequence analysis which extracted from rice seedlings whose seeds had been treated with 1000 µMGA or water prior to germination.

Gene	FDR (DESeq stats P<0.05 after correction)	Log2 Fold Change (DESeq stats p<0.05 after correction)	Description
Os03g0757600	0.035	-7.82	UDP-glucuronosyl/UDP-glucosyltransferase family protein / Similar to Indole-3-acetate beta-glucosyltransferase.
Os04g0612500	0.035	1.38	Similar to 36.4 kDa proline-rich protein.
Os06g0512700	0.0350	-6.00	Thionin
Os08g0163800	5×10^{-8}	-21.39	Similar to Anti-silencing protein 1.
Os10g0416500	1×10^{-4}	-2.35	Similar to Chitinase 1 precursor (EC 3.2.1.14) (Tulip bulb chitinase-1) (TBC-1).
Os12g0227500	0.035	-6.30	Similar to Beta-glucosidase aggregating factor.

5.4. Discussion

The aim of the chapter was to identify changes in the expression of genes in seedlings whose seeds had been soaked in 1000 µM GA3 prior to germination. This was to further understand the genetic regulation of seedling growth as a result of this treatment and the interaction of gene expression with the PGR. Due to high duplication rates in the samples, it proved difficult to obtain a large number of differentially expressed genes (Table 5.5). The one gene upregulated in the seedlings whose seeds were treated with 1000 µM GA3 - *Os04g0612500*, is similar to a proline-rich protein (Table 5.5). Proline is a proteogenic amino acid which has an important role in plant growth and differentiation, including protein synthesis, osmotic adjustment, cell wall synthesis and protection of protein degradation under stress. During the seed germination proline regulates cyclin gene functions as part of cell cycling (Kishor *et al.*, 2015). Together with its role in cell wall synthesis, the upregulation of this gene might be related to the high rate of growth and development observed in seedlings whose seeds were soaked in GA. Proline-rich proteins (PRP) are plant structural cell wall proteins, which have crucial roles in the maintenance of proteins in the plant cell wall during plant development and stress-induced fortification by maintaining the plasma membrane factors (Stein *et al.*, 2011, Chen & Varner, 1985, Tierney *et al.*, 1988). Seedling growth and root nodule formation have previously been associated with the expression of PRP genes (Santino *et al.*, 1997). However, the precise functions of PRPs are still under investigation.

The remaining five differentially expressed genes were downregulated in the seedlings whose seeds were soaked in 1000 µM GA3 relative to those whose seeds were soaked in water (Table 5.5). The most downregulated gene, *Os08g0163800*, encodes an ANTI-SILENCING FUNCTION1 (ASF1) protein which is controlling H3 and H4 histone types. Controlling the H3 and H4 occurs by N terminal tail which is very important structure chemically and for signalling process of control the genes regulation. DNA replication, transcription and translation cannot be happened without unwrapping the DNA from histone proteins (Li *et al.*, 2008, Lario *et al.*, 2013). There is no obvious link to the downregulation of this gene and treatment of the seed with GA3.

The second most downregulated gene *Os03g0757600* from glucosyltransferase family protein. Glycosyltransferases are an enormous family linked with transfer of sugars and initiating glycosidic bonds which are a covalent bond that hold the glycoside together. UDP-glycosyltransferases family (UGTs) plays crucial roles in plant growth and their response to biotic and abiotic stress by catalysing the transfer of the monosaccharide through glycosylation. Glycosylation mediated by Family-1 UDP-glycosyltransferases (UGTs) plays crucial roles in plant growth and adaptation to various stress conditions (Lu *et al.*, 2013, Li *et al.*, 2014, Zhang *et al.*, 2018). Control of plant growth and development occurs by regulating the PGRs inside the plant cell. Regulation of PGRs can be achieved when sugars are conjugated with PGRs, amino acids or proteins (Ostrowski & Jakubowska, 2014). UGTs have previously been identified that add glucose to gibberellin to form GA-glucose conjugates (Ostrowski & Jakubowska, 2014). The down regulation of this gene here might be linked to a reduction in this process in the shoots of seedlings whose seeds were soaked in GA3.

The third most downregulated gene was *Os12g0227500* which is similar to Beta-glucosidase aggregating factor. The hydrolysis of β-glycosidic bonds of aryl and alkyl esters is catalyzed by β-Glucosidases (3.2.1.21) (Cicek & Esen, 1998, Morant *et al.*, 2008). Two type of β-glucosidase proteins bglu1 and bglu2 were cloned and sequenced from rice seedlings. During germination both bglu1 and bglu2 are highly expressed in shoots compared with roots and seeds. This expression of the genes encoding of bglu1 and bglu2 at the seedling stage implies the role of these β-glucosidase in recycling the saccharides, cell division and cell expansion (Opassiri *et al.*, 2003). The results suggested that bglu1 role is to recycle the remaining saccharides from the cell wall to generate new oligosaccharides (Opassiri *et al.*, 2003). In conjunction with PGRs, bglu1 was inhibited by IAA and GA3 by 54% and 24%, respectively. This inhibition may explained through, PGRs and BGlu1 compete to bind to the active site such as aglycone binding site (Opassiri *et al.*, 2003). However, bglu1 and bglu2 expression in barley were decreased (Leah *et al.*, 1995). Since the development of seedlings whose seeds were treated with GA was quicker than those whose seeds were treated with water, the expression of this gene may be relatively high still in the seedlings of water treated seed and decreasing in the seedlings of GA treated seed. However, the function of *Os12g0227500* gene is unclear, thus, GenBank database was searched for sequences similar to Beta-glucosidase aggregating factor. The b-glucosidase-aggregating factor was described as a 35-kDA protein in a study conducted on two genotypes of (*Zea mays* L.) wild-type (K55) and (H95), which mediate the b-glucosidase activity (Esen & Blanchard, 2000).

The fourth downregulated gene was *Os06g0512700*. This gene encodes thionin which is an antimicrobial and well-known for plant defence roles. However, the expression of rice thionin genes are regulated by PGRs (Iwai *et al.*, 2002, Kitanaga *et al.*, 2006, Ji *et al.*, 2015). Rice thionin genes belong to a multi-gene family consisting of 12 members, which are all found to be in a specific region of chromosome 6 (Kitanaga *et al.*, 2006). In a study conducted by Kitanaga *et al.* (2006) they observed crosstalk between jasmonic acid (JA) and thionin gene expression. This regulation occurs when GAs and brassinosteroids work synergistically to regulate JA concentrations which increased thionin gene expression and controlled rice seedling growth and development. It might be expected that treating seeds with GA, could increase JA and therefore thionin gene expression in this study, but this was not observed as this gene was downregulated in seedlings whose seed were treated with GA (Table 5.5).

The last downregulated gene was *Os10g0416500* which is similar to Chitinase 1 precursor. Chitinases (E.C 3.2.2.14) are antifungal defence proteins which play a key role in a nonspecific plant defence response. Chitinases are encoded by small gene families (Graham & Sticklen, 1994). The activity of chitinase gene expression and enzymes vary according to plant developmental stages and plant tissues such as shoot, root, seeds and flowers. However, chitinase concentration found to be significantly concentrated in root and flowers (Hermans *et al.*, 2010). This may be explained through the defence role of chitinases in protecting the plant meristems (Graham & Sticklen, 1994, Eckardt, 2008).

Chapter 6. Final Discussion

The aim of this thesis was to investigate the effect of soaking rice seeds in different PGRs and different concentrations on the growth and development under the well-watered and drought treatments. Further investigation of this research was conducted to identify the genes and the gene expression differences between the seedlings whose seeds were soaked in 1000 µM GA3 and seedings whose seeds were soaked in water. The effect of PGRs on rice genotypes were investigated up to the seedling stage and to maturity under controlled environment conditions. The results were encouraging, showing some additional root biomass at depth which may benefit the plant at later stages of growth and under drought stress. However, the relationship between PGRs, drought stress, genotypes and gene expression is still not fully resolved and further research can be undertaken in this area. These are linked to the overall objective of reducing the amount of water used by the rice crop. Therefore, this discussion will focus on using PGRs and their effects on root system under the drought stress and the genes related to this.

Water is a key driver for agricultural sustainability. Crop production is seriously limited by water scarcity worldwide. Water use by agriculture is estimated to be on average 69% of the abstracted water. Therefore, reducing the amount of water used by agriculture can have a potential to save up to 35% of global freshwater resources (Cosgrove & Rijsberman, 2014). Transgenic approaches, plant breeding to use less water by having longer root systems to access water at depth, cultural techniques such as how soil is prepared and using chemical enhancement such as PGRs are different methods which used to alter root distribution in the soil to promote water capture (Ferguson & Lessenger, 2006, Joo *et al.*, 2013, Farooq *et al.*, 2019). However, the practical applications of using PGRs, improving both rice seedling

emergence and crop performance under biotic and abiotic stress, maintenance of early establishment of uniform seedling and enhanced competition with weeds, also results in reduced labour cost for seedling transplanting and water use (Bari & Jones, 2009, Zhang *et al.*, 2009, Fahad *et al.*, 2016, Lamichhanea *et al.*, 2019). The reduced labour cost is associated with seedbed preparation which is normally required for planted rice, when pre-germinated rice seedling are transferred to the paddy field (Farooq *et al.*, 2011b). Consequently, rice seedling emergence and crop performance under biotic and abiotic stress is improved.

6.1. The response of rice seedlings to PGRs

The use of PGRs as seed priming agents was investigated in this study to test the hypothesis that, root system growth can be promoted, which can improve establishment at the early stages of plant growth, and could result in increased plant resistance to drought stress at late growth stages. Results demonstrated that, seedlings of seeds treated with PGRs showed a significant response with both positive and negative effects on root and shoot system (Figures 3.6; 3.7; 3.10; 3.14; 3.16; 3.20; 3.23; 3.30). Among the twenty rice genotypes whose seeds were soaked in 1000 µM GA3, the root system of genotype GHRAIBA was significantly ($P<0.001$) increased (Figure 3.16). Although, there were a few other genotypes that showed significant ($P<0.001$) responses to 1000 µM GA3. Consequently, GHRAIBA was chosen for further study as it is also one of the genotypes obtained from arid and/or semi-arid rice growing regions. The use of PGRs as seed priming agents used in this study, are not only a cost-effective methodology and a practical solution for farmers, but also could contribute to reducing the gap between potential and actual yields by reducing the negative effects of the biotic and abiotic stresses.

The considerable advantages of seed priming can be varied between improving the seedling early establishment which results in increase the photosynthetic rate, strengthen the plant antioxidant system, earlier flowering and improved crop performance (Farooq *et al.*, 2019).

6.2. Variability in response to PGRs within rice varieties

The variation in response of different rice genotypes to seed treatments with PGRs confirmed the ability of PGRs to manipulate seedling growth and development. PGRs stimulated the early increase in root system during the early stages of the plant growth and establishing a longer root system, which in GHRAIBA genotype was up to 6 cm longer compared to the control (Figures 3.16).

6.3. The impact of GA3 seed treatment on growth, development and yield

The increase in root system for GHRAIBA genotype, up to the seedling stage, was investigated further in soil. In the soil experiment, which was conducted to investigate the hypothesis of using PGRs as seed priming agents to provide a long-term effect on the shoot and root system for the plant whose seeds were soaked in PGRs. The drought stress was imposed by using a new irrigation system which was built for this purpose (Figure 4.4). The drought stress was chosen based on many preliminary experiments to be at 70% of the field capacity. However, shoot biomass results showed no significant ($P>0.05$) difference between plants whose seeds were treated with 1000 µM GA3 and plants whose seeds treated with water. Consequently, imposing more drought stress may reveal the answer of whether GA3 would be able to modify the drought response of rice plants whose seeds were treated with GA3.

Results showed at the early stage of germination, 4 DAS, GA3 treatment increased the seed germination percentage significantly ($P<0.001$). As well, seedling dry weight was significantly ($P<0.05$) increased after 14 DAS. These

results confirmed the ability of the priming seeds with GA3 to modify the plant growth and development at the early stages.

However, the effect of treating seeds with GA3 was not clear 40 DAS when the shoot and root system from harvested plants were analysed. Interestingly, at the maturity (139 DAS), there was a significant ($P<0.001$) effect of treating seeds with GA3 on the root fresh and dry weight for the lowest part of the root system (P4) (Figure 4.17).

The effect of GA seed treatment on the plant at the germination, seedling and maturity growth stages, might be explained through, plants whose seeds were treated with 1000 µM GA3 had established a longer root system at the early growth stage which was reflected in the amount of root system found in the lower parts of the root system (P4) at maturity. These results agreed with previous research that suggests early establishment of long and strong root system could have a significant modification to the root length and strength at the later plant growth and development especially, under stressed environments such as drought stress (Ingram & Malamy, 2010, Rich & Watt, 2013).

However, 40 DAS there was no significant ($P<0.001$) difference of reducing the amount of water by 30% on the shoot fresh weight, tiller number, root fresh weight and root dry weight. These results imply that during the early stages of the plant growth and development, 30% of water for irrigation could be saved. However, these results need more confirmation under the field conditions. At the maturity (139 DAS), there was a significant ($P<0.05$) difference between well-watered and drought treatment on shoot fresh weight and the 100 seeds weight. This implies that, water availability became more essential for plant growth and development during reproductive phase and ripening phase (Figure 4.3). When these data are linked to

growing rice in the field for arid and semi-arid regions, saving water at the early stage of rice growth and development is challenging.

This is because the rice growing time occurs between June and November, when rainfall is low and temperatures are highest in June and July (Prasad *et al.*, 2006a).

6.4. The effect of PGRs on the gene and the gene expression.

The analysis of gene expression changes in seedlings whose seeds were treated with 1000 µM GA3 showed only six genes were differentially expressed compared to seedlings whose seeds were soaked in water. This is potentially due to issues in the library preparation phase, which resulted in unusually high duplication rates for the samples (Table 5.5). Only one of the six genes was upregulated for seedlings whose seeds were treated with 1000 µM GA3. Interestingly, this *Os04g0612500* gene was described to have an important role in cell wall synthesis, cell cycling and protection of protein degradation under stress (Kishor *et al.*, 2015). The upregulation of this gene might be explained through, the treatment of seeds with GA prior to germination, stimulating faster seedling growth. During seed germination and seedling growth, tissue GA concentrations are high (Sun, 2010, Binenbaum *et al.*, 2018). These high concentrations of GA might trigger the *Os04g0612500* gene to be highly expressed. However, the relationship between PGRs and gene expression still requires further research.

This relationship becomes more complex under stress environments. Therefore, investigating the effect of using single PGR or a combination of PGRs with different concentrations at different plant growth stages may identify some of these complex interactions between gene expression regulated by PGRs and the expression of genes related to plant stress.

6.5. Study's limitations

Since rice cannot be planted in the open field under the UK environment, growing rice under controlled-environment or glasshouse and finding the exact conditions to reflect the field growing rice conditions was the main challenge for this study. In addition, the size of the conducted experiments was restricted by the size of these facilities. These restrictions limited the investigation of multiple genotypes, more tubes, and other treatment combinations e.g. GA and NAA in plants grown to maturity.

The number of soil moisture sensors was another limiting factor for this study. The reduced number of sensors meant that soil moisture could only be monitored at two depths in a few tubes. Consequently, this may have reduced the ability to control the soil moisture accurately across the experiment. Whilst some effects of drought were observed in the biomass and growth of the plants, they were not as extreme as those observed in preliminary experiments. Consequently, it was not possible to determine if the treatment of seeds with GA3 could improve plant tolerance to drought stress through improving root development at depth.

6.6. Future suggested work

This study has highlighted the differences in response of root traits at early seedling stage and between different rice genotypes to soaking of seeds with different PGRs at different concentrations. Soaking seeds in PGRs could have the benefits of reducing the cost of the transplanting for the rice seedlings. Phenological and physiological acceleration for primed field-sown crops becomes necessary under water deficit. This acceleration of phenology and physiology in early establishment results in a longer root system to capture more water and nutrient resources at depth. However, only one genotype was investigated to maturity under controlled-environment and drought conditions.

More investigations into priming seeds with different PGRs and different concentrations or application at different growth stages of plant development and in response to drought, may have the potential to reveal the drought response mechanism within the plant and improve the physiological adaptation of the root system to better cope with drought stress. This could include the use of combinations of PGRs which might work synergistically to improve growth and drought tolerance. Further work is necessary to establish a stronger drought stress and determine if seed priming with GA3 can improved drought tolerance in rice plants.

Linked with the genetic response in terms of genes and their expression for each growth stage in seedlings and plants whose seeds have been primed with different PGRs would further our understanding of the signalling mechanisms associated with these PGRs and uncover the complexity of drought response mechanism. Further research is needed to demonstrate the effects of this approach, both in controlled conditions and in the field, on improving drought tolerance in rice.

References

ABELES, F. B., MORGAN, P. W. & SALTVEIT, M. E., JR. 1992. *Ethylene in plant biology, Second edition*, Academic Press, London.

AFZAL, I., BASRA, S. M., AHMAD, N., CHEEMA, M. A., WARRAICH, E. A. & KHALIQ, A. 2002. Effect of priming and growth regulator treatments on emergence and seedling growth of hybrid maize (*Zea mays* L.). *International Journal of Agriculture and Biology*, 4, 303-306.

AIKEN, R. & SMUCKER, A. 1996. Root system regulation of whole plant growth. *Annual Review of Phytopathology*, 34, 325-346.

AKRAM, H., ALI, A., SATTAR, A., REHMAN, H. & BIBI, A. 2013. Impact of water deficit stress on various physiological and agronomic traits of three basmati rice (*Oryza sativa* L.) cultivars. *Journal of Animal and Plant Sciences*, 23, 1415-1423.

ALBACETE, A. A., MARTÍNEZ-ANDÚJAR, C. & PÉREZ-ALFOCEA, F. 2014. Hormonal and metabolic regulation of source–sink relations under salinity and drought: from plant survival to crop yield stability. *Biotechnology Advances*, 32, 12-30.

ANURADHA, S. & RAO, S. S. R. 2003. Application of brassinosteroids to rice seeds (*Oryza sativa* L.) reduced the impact of salt stress on growth, prevented photosynthetic pigment loss and increased nitrate reductase activity. *Plant Growth Regulation*, 40, 29-32.

ARITE, T., UMEHARA, M., ISHIKAWA, S., HANADA, A., MAEKAWA, M., YAMAGUCHI, S. & KYOZUKA, J. 2009. d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant and Cell Physiology*, 50, 1416-1424.

ATKINSON, J. A., RASMUSSEN, A., TRAINI, R., VOß, U., STURROCK, C., MOONEY, S. J., WELLS, D. M. & BENNETT, M. J. 2014. Branching out in roots: uncovering form, function, and regulation. *Plant Physiology*, 166, 538-550.

BABU, R. C., NGUYEN, B. D., CHAMARERK, V., SHANMUGASUNDARAM, P., CHEZHIAN, P., JEYAPRAKASH, P., GANESH, S., PALCHAMY, A., SADASIVAM, S. & SARKARUNG, S. 2003. Genetic analysis of drought resistance in rice by molecular markers. *Crop Science*, 43, 1457-1469.

BAI, C., LIANG, Y. & HAWKESFORD, M. J. 2013. Identification of QTLs associated with seedling root traits and their correlation with plant height in wheat. *Journal of Experimental Botany*, 64, 1745-1753.

BAO, Y., AGGARWAL, P., ROBBINS, N. E., STURROCK, C. J., THOMPSON, M. C., TAN, H. Q., THAM, C., DUAN, L., RODRIGUEZ, P. L. & VERNOUX, T. 2014. Plant roots use a patterning mechanism to position lateral root branches toward available water. *Proceedings of the National Academy of Sciences USA*, 111, 9319-9324.

BARI, R. & JONES, J. D. 2009. Role of plant hormones in plant defence responses. *Plant Molecular Biology*, 69, 473-488.

BARKER, T., CAMPOS, H., COOPER, M., DOLAN, D., EDMEADES, G., HABBEN, J., SCHUSSLER, J., WRIGHT, D. & ZINSELMEIER, C. 2005. Improving drought tolerance in maize. *Plant Breeding Reviews*, 25, 173-253.

BEWLEY, J. D. 2001. Seed Germination and Reserve Mobilization. In *Encyclopedia of Life Sciences, Wiley & Sons,* doi:10.1038/npg.els.0002047.

BEWLEY, J. D. & MARCUS, A. 1990. Gene expression in seed development and germination. *Progress In Nucleic Acid Research And Molecular Biology*, 38, 165-193.

BINENBAUM, J., WEINSTAIN, R. & SHANI, E. 2018. Gibberellin localization and transport in plants. *Trends in Plant Science*, 23, 410-421.

BISHOPP, A. & LYNCH, J. P. 2015. The hidden half of crop yields. *Nature Plants*, 1, 15117.

BLAKESLEE, J. J., PEER, W. A. & MURPHY, A. S. 2005. Auxin transport. *Current Opinion in Plant Biology*, 8, 494-500.

BLUM, A. 2005. Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research*, 56, 1159-1168.

BLUM, A. 2009. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Research*, 112, 119-123.

BOTHA, F., POTGIETER, G. & BOTHA, A.-M. 1992. Respiratory metabolism and gene expression during seed germination. *Plant Growth Regulation*, 11, 211-224.

BOYER, J. S. 1982. Plant productivity and environment. *Science*, 218, 443-448.

CAMPOS, H., COOPER, M., HABBEN, J., EDMEADES, G. & SCHUSSLER, J. 2004. Improving drought tolerance in maize: a view from industry. *Field Crops Research*, 90, 19-34.

CELENZA, J., GRISAFI, P. L. & FINK, G. R. 1995. A pathway for lateral root formation in *Arabidopsis thaliana*. *Genes & development*, 9, 2131-2142.

CHEN, D., WANG, S., CAO, B., CAO, D., LENG, G., LI, H., YIN, L., SHAN, L. & DENG, X. 2016. Genotypic variation in growth and physiological response to drought stress and re-watering reveals the critical role of recovery in drought adaptation in maize seedlings. *Frontiers in Plant Science*, 6, 1241.

CHEN, J. & VARNER, J. E. 1985. Isolation and characterization of cDNA clones for carrot extensin and a proline-rich 33-kDa protein. *Proceedings of the National Academy of Sciences*, 82, 4399-4403.

CHOUDHARY, A. K. & SURI, V. 2014. Integrated nutrient-management technology for direct-seeded upland rice (*Oryza sativa* L.) in Northwestern Himalayas. *Communications in Soil Science and Plant Analysis*, 45, 777-784.

CHUN, S.-C., SCHNEIDER, R. & COHN, M. 1997. Sodium hypochlorite: effect of solution pH on rice seed disinfestation and its direct effect on seedling growth. *Plant Disease*, 81, 821-824.

CICEK, M. & ESEN, A. 1998. Structure and expression of a dhurrinase (β-glucosidase) from sorghum. *Plant Physiology*, 116, 1469-1478.

Clarke, C. 2017. *Quantifying rooting at depth in a wheat doubled haploid population with introgression from wild emmer*. PhD thesis, University of Reading, Reading.

CLARK, R. T., FAMOSO, A. N., ZHAO, K., SHAFF, J. E., CRAFT, E. J., BUSTAMANTE, C. D., MCCOUCH, S. R., ANESHANSLEY, D. J. & KOCHIAN, L. V. 2013. High-throughput two-dimensional root system phenotyping platform facilitates genetic analysis of root growth and development. *Plant, Cell & Environment*, 36, 454-466.

CLARK, R. T., MACCURDY, R. B., JUNG, J. K., SHAFF, J. E., MCCOUCH, S. R., ANESHANSLEY, D. J. & KOCHIAN, L. V. 2011. Three-dimensional root phenotyping with a novel imaging and software platform. *Plant Physiology*, 156, 455-465.

COLEBROOK, E. H., THOMAS, S. G., PHILLIPS, A. L. & HEDDEN, P. 2014. The role of gibberellin signalling in plant responses to abiotic stress. *Journal of experimental biology*, 217, 67-75.

COMAS, L., BECKER, S., CRUZ, V. M. V., BYRNE, P. F. & DIERIG, D. A. 2013. Root traits contributing to plant productivity under drought. *Frontiers in Plant Science*, 4, 442.

CONNOR, R., RENATA, A., ORTIGARA, C., KONCAGÜL, E., UHLENBROOK, S., LAMIZANA-DIALLO, B. M., ZADEH, S. M., QADIR, M., KJELLÉN, M. & SJÖDIN, J. 2017. The United Nations World Water Development Report 2017. Wastewater: The Untapped Resource. *The United Nations World Water Development Report*.

COSGROVE, W. J. & RIJSBERMAN, F. R. 2014. World water vision: making water everybody's business, Routledge.

CUTLER, S. R., RODRIGUEZ, P. L., FINKELSTEIN, R. R. & ABRAMS, S. R. 2010. Abscisic acid: emergence of a core signaling network. *Annual Review of Plant Biology*, 61, 651-679.

DAVIES, P. J. 2010. The plant hormones: their nature, occurrence, and functions. In: Davies P. J. (ed) *Plant Hormones*. Springer, Dordrecht.

DE DORLODOT, S., FORSTER, B., PAGÈS, L., PRICE, A., TUBEROSA, R. & DRAYE, X. 2007. Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends in Plant Science*, 12, 474-481.

DE SMET, I., WHITE, P. J., BENGOUGH, A. G., DUPUY, L., PARIZOT, B., CASIMIRO, I., HEIDSTRA, R., LASKOWSKI, M., LEPETIT, M. & HOCHHOLDINGER, F. DRAYE, X., ZHANG, H., BROADLEY, M. R., PERET, B., HAMMOND, J. P., FUKAKI, H., MOONEY, S., LYNCH, J. P., NACRY, P., SCHURR, U., LAPLAZE, L., BENFEY, P., BEECKMAN, T. & BENNETT, M. 2012. Analyzing lateral root development: how to move forward. *The Plant Cell,* 24**,** 15-20.

DEKKERS, B. J., HE, H., HANSON, J., WILLEMS, L. A., JAMAR, D. C., CUEFF, G., RAJJOU, L., HILHORST, H. W. & BENTSINK, L. 2016. The Arabidopsis DELAY OF GERMINATION 1 gene affects ABSCISIC ACID INSENSITIVE 5 (ABI 5) expression and genetically interacts with ABI 3 during Arabidopsis seed development. *The Plant Journal*, 85, 451-465.

DEN HERDER, G., VAN ISTERDAEL, G., BEECKMAN, T. & DE SMET, I. 2010. The roots of a new green revolution. *Trends in Plant Science*, 15, 600-607.

DI GIROLAMO, G. & BARBANTI, L. 2012. Treatment conditions and biochemical processes influencing seed priming effectiveness. *Italian Journal of Agronomy*, e25-e25.

DOBERMANN, A. & FAIRHURST, T. 2000. Rice: nutrient disorders & nutrient management, International Rice Research Institute, Philippines.

DUBOIS, M., VAN DEN BROECK, L. & INZÉ, D. 2018. The pivotal role of ethylene in plant growth. *Trends in Plant Science*, 23, 311-323.

EAPEN, D., BARROSO, M. L., CAMPOS, M. E., PONCE, G., CORKIDI, G., DUBROVSKY, J. G. & CASSAB, G. I. 2003. A no hydrotropic response root mutant that responds positively to gravitropism in Arabidopsis. *Plant Physiology*, 131, 536-546.

EAPEN, D., BARROSO, M. L., PONCE, G., CAMPOS, M. E. & CASSAB, G. I. 2005. Hydrotropism: root growth responses to water. *Trends in Plant Science*, 10, 44-50.

ECKARDT, N. A. 2008. Chitin signaling in plants: insights into the perception of fungal pathogens and rhizobacterial symbionts. *Plant Cell*, 20, 241-243. doi: 10.1105/tpc.108.058784.

EHLERS, W. & GOSS, M. 2016. Water dynamics in plant production, CABI.

EHRENSHAFT, M. & BRAMBL, R. 1990. Respiration and mitochondrial biogenesis in germinating embryos of maize. *Plant Physiology*, 93, 295-304.

ELLIOTT, J., DERYNG, D., MÜLLER, C., FRIELER, K., KONZMANN, M., GERTEN, D., GLOTTER, M., FLÖRKE, M., WADA, Y. & BEST, N. 2014. Constraints and potentials of future irrigation water availability on agricultural production under climate change. *Proceedings of the National Academy of Sciences USA*, 111, 3239-3244.

ESEN, A. & BLANCHARD, D. J. 2000. A specific β-glucosidase-aggregating factor is responsible for the β-glucosidase null phenotype in maize. *Plant Physiology*, 122, 563-572.

EVANS, R. G. & SADLER, E. J. 2008. Methods and technologies to improve efficiency of water use. *Water Resources Research,* 44, W00E04.

FADER, M., SHI, S., BLOH, W. V., BONDEAU, A. & CRAMER, W. 2016. Mediterranean irrigation under climate change: more efficient irrigation needed to compensate for increases in irrigation water requirements. *Hydrology and Earth System Sciences*, 20, 953-973.

FAHAD, S., HUSSAIN, S., BANO, A., SAUD, S., HASSAN, S., SHAN, D., KHAN, F. A., KHAN, F., CHEN, Y. & WU, C. 2015. Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. *Environmental Science and Pollution Research*, 22, 4907-4921.

FAHAD, S., HUSSAIN, S., SAUD, S., HASSAN, S., IHSAN, Z., SHAH, A. N., WU, C., YOUSAF, M., NASIM, W. & ALHARBY, H. 2016. Exogenously applied plant growth regulators enhance the morpho-physiological growth and yield of rice under high temperature. *Frontiers in Plant Science*, 7, 1250.

FAO. 2015. Water withdrawal by sector. Aquastat. Retrieved on 6th January 2020 from, http://www.fao.org/nr/water/aquastat/water_use/index.stm. Date accessed 15/11/2017.

FAROOQ, M., ATIQUE-UR-REHMAN, AZIZ, T. & HABIB, M. 2011a. Boron nutripriming improves the germination and early seedling growth of rice (*Oryza sativa* L.). *Journal of plant nutrition*, 34, 1507-1515.

FAROOQ, M., BASRA, S., AFZAL, I. & KHALIQ, A. 2006. Optimization of hydropriming techniques for rice seed invigoration. *Seed Science and Technology*, 34, 507-512.

FAROOQ, M., BASRA, S., WAHID, A., AHMAD, N. & SALEEM, B. 2009a. Improving the drought tolerance in rice *(Oryza sativa* L.) by exogenous application of salicylic acid. *Journal of Agronomy and Crop Science*, 195, 237-246.

FAROOQ, M., BASRA, S., WAHID, A., KHALIQ, A. & KOBAYASHI, N. 2009b. Rice seed invigoration: a review. In LICHTFOUSE, E. (ed) *Organic farming, pest control and remediation of soil pollutants*, Springer, Dordrecht.

FAROOQ, M., SIDDIQUE, K. H., REHMAN, H., AZIZ, T., LEE, D.-J. & WAHID, A. 2011b. Rice direct seeding: experiences, challenges and opportunities. *Soil and Tillage Research*, 111, 87-98.

FAROOQ, M., USMAN, M., NADEEM, F., UR REHMAN, H., WAHID, A., BASRA, S. M. & SIDDIQUE, K. H. 2019. Seed priming in field crops: potential benefits, adoption and challenges. *Crop and Pasture Science*, 70, 731-771.

FAROOQ, M., WAHID, A., KOBAYASHI, N., FUJITA, D. & BASRA, S. M. A. 2009c. Plant drought stress: effects, mechanisms and management. *Agronomy for Sustainable Development*, 29, 185-212.

FAROOQ, M., WAHID, A. & SIDDIQUE, K. H. 2012. Micronutrient application through seed treatments: a review. *Journal of soil science and plant nutrition*, 12, 125-142.

FELDMAN, L. J. 1984. Regulation of root development. *Annual Review of Plant Physiology*, 35, 223-242.

FERGUSON, L. & LESSENGER, J. E. 2006. Plant growth regulators. In *Agricultural Medicine*. *A Practical Guide,* Lessenger, J.E. (Ed.). Springer, pp. 156-166.

FINKELSTEIN, R. 2013. Abscisic acid synthesis and response. *Arabidopsis book* 11: e0166. doi.org/10.1199/tab.0166.

FLEURY, D., JEFFERIES, S., KUCHEL, H. & LANGRIDGE, P. 2010. Genetic and genomic tools to improve drought tolerance in wheat. *Journal of Experimental Botany*, 61, 3211-3222.

FRÉBORT, I., KOWALSKA, M., HLUSKA, T., FRÉBORTOVÁ, J. & GALUSZKA, P. 2011. Evolution of cytokinin biosynthesis and degradation. *Journal of Experimental Botany*, 62, 2431-2452.

FUJII, H. & ZHU, J.-K. 2009. Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction, and stress. *Proceedings of the National Academy of Sciences USA*, 106, 8380-8385.

GEISLER, M., BLAKESLEE, J. J., BOUCHARD, R., LEE, O. R., VINCENZETTI, V., BANDYOPADHYAY, A., TITAPIWATANAKUN, B., PEER, W. A., BAILLY, A., RICHARDS, E. L., EJENDAL, K. F. K., SMITH, A. P., BAROUX, C., GROSSNIKLAUS, U., MÜLLER, A., HRYCYNA, C. A., DUDLER, R., MURPHY, A. S. & MARTINOIA, E. 2005. Cellular efflux of auxin catalyzed by the Arabidopsis MDR/PGP transporter AtPGP1. *The Plant Journal*, 44, 179-194.

GIRON, D., FRAGO, E., GLEVAREC, G., PIETERSE, C. M. & DICKE, M. 2013. Cytokinins as key regulators in plant–microbe–insect interactions: connecting plant growth and defence. *Functional Ecology*, 27, 599-609.

GORNALL, J., BETTS, R., BURKE, E., CLARK, R., CAMP, J., WILLETT, K. & WILTSHIRE, A. 2010. Implications of climate change for agricultural productivity in the early twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 2973-2989.

GOU, J., STRAUSS, S. H., TSAI, C. J., FANG, K., CHEN, Y., JIANG, X. & BUSOV, V. B. 2010. Gibberellins regulate lateral root formation in Populus through interactions with auxin and other hormones. *The Plant Cell*, 22, 623-639.

GRAHAM, L. S. & STICKLEN, M. B. 1994. Plant chitinases. *Canadian Journal of Botany*, 72, 1057-1083.

GREENWOOD, D., ZHANG, K., HILTON, H. & THOMPSON, A. 2010. Opportunities for improving irrigation efficiency with quantitative models, soil water sensors and wireless technology. *The Journal of Agricultural Science*, 148, 1-16.

GREGORY, P. J. 2008. *Plant roots: growth, activity and interactions with the soil*, John Wiley & Sons, London.

GUO, J., WU, J., JI, Q., WANG, C., LUO, L., YUAN, Y., WANG, Y. & WANG, J. 2008. Genome-wide analysis of heat shock transcription factor families in rice and Arabidopsis. *Journal of Genetics and Genomics*, 35, 105-118.

HAN, C. & YANG, P. 2015. Studies on the molecular mechanisms of seed germination. *Proteomics*, 15, 1671-1679.

HAN, H. & YANG, W. 2009. Influence of uniconazole and plant density on nitrogen content and grain quality in winter wheat in South China. *Plant Soil Environment*, 55, 159-66.

HEDDEN, P. & SPONSEL, V. 2015. A Century of Gibberellin Research. *Journal of Plant Growth Regulation*, 34, 740-760.

HENRY, A. 2013. IRRI's drought stress research in rice with emphasis on roots: accomplishments over the last 50 years. *Plant Root*, 7, 92-106.

HENRY, A. & HARDY, B. 2012. Methodologies for root drought studies in rice. *International Rice Research Institute* (IRRI).

HERMAN, E., BAUMGARTNER, B. & CHRISPEELS, M. 1981. Uptake and apparent digestion of cytoplasmic organelles by protein bodies (protein storage vacuoles) in mung bean cotyledons. *European journal of cell biology*, 24, 226-235.

HERMANS, C., PORCO, S., VERBRUGGEN, N. & BUSH, D. R. 2010. Chitinase-like protein CTL1 plays a role in altering root system architecture in response to multiple environmental conditions. *Plant Physiology*, 152, 904-917.

HOCHHOLDINGER, F. & TUBEROSA, R. 2009. Genetic and genomic dissection of maize root development and architecture. *Current Opinion in Plant Biology*, 12, 172-177.

HRUZ, T., LAULE, O., SZABO, G., WESSENDORP, F., BLEULER, S., OERTLE, L., WIDMAYER, P., GRUISSEM, W. & ZIMMERMANN, P. 2008. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Advances in Bioinformatics*, 2008. doi:10.1155/2008/420747.

HSIAO, T. C. 1973. Plant responses to water stress. *Annual Review of Plant Physiology*, 24, 519-570.

HU, H., DAI, M., YAO, J., XIAO, B., LI, X., ZHANG, Q. & XIONG, L. 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceedings of the National Academy of Sciences USA*, 103, 12987-12992.

HUBBARD, K. E., NISHIMURA, N., HITOMI, K., GETZOFF, E. D. & SCHROEDER, J. I. 2010. Early abscisic acid signal transduction mechanisms: newly discovered components and newly emerging questions. *Genes & development*, 24, 1695-1708.

INGRAM, P. A. & Malamy J. E. 2010. Root system architecture. In Kader J. C. & Delseny, M. (eds) *Advances in Botanical Research*. pp 75–117.

IQBAL, M. & ASHRAF, M. 2013. Alleviation of salinity-induced perturbations in ionic and hormonal concentrations in spring wheat through seed preconditioning in synthetic auxins. *Acta Physiologiae Plantarum*, 35, 1093-1112.

IQBAL, N., NAZAR, R., KHAN, M. I. R., MASOOD, A. & KHAN, N. A. 2011. Role of gibberellins in regulation of source-sink relations under optimal and limiting environmental conditions. *Current Science*, 100, 998-1007.

IRRI, A. 2010. CIAT (2010) Global Rice Science Partnership (GRiSP). International Rice Research Institute, Los Baños, Philippines.

ITO, Y., KATSURA, K., MARUYAMA, K., TAJI, T., KOBAYASHI, M., SEKI, M., SHINOZAKI, K. & YAMAGUCHI-SHINOZAKI, K. 2006. Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant and Cell Physiology*, 47, 141-153.

ITOH, J.-I., NONOMURA, K.-I., IKEDA, K., YAMAKI, S., INUKAI, Y., YAMAGISHI, H., KITANO, H. & NAGATO, Y. 2005. Rice plant development: from zygote to spikelet. *Plant and Cell Physiology*, 46, 23-47.

IWAI, T., KAKU, H., HONKURA, R., NAKAMURA, S., OCHIAI, H., SASAKI, T. & OHASHI, Y. 2002. Enhanced resistance to seed-transmitted bacterial diseases in transgenic rice plants overproducing an oat cell-wall-bound thionin. *Molecular Plant-Microbe Interactions*, 15, 515-521.

JAGADISH, S., CRAUFURD, P. & WHEELER, T. 2008. Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. Crop Science, 48, 1140-1146.

JAGADISH, S., MUTHURAJAN, R., OANE, R., WHEELER, T. R., HEUER, S., BENNETT, J. & CRAUFURD, P. Q. 2010. Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *Journal of Experimental Botany*, 61, 143-156.

JALEEL, C. A., MANIVANNAN, P., WAHID, A., FAROOQ, M., AL-JUBURI, H. J., SOMASUNDARAM, R. & PANNEERSELVAM, R. 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. *International Journal of Agriculture and Biology*, 11, 100-105.

JEONG, J. S., KIM, Y. S., BAEK, K. H., JUNG, H., HA, S.-H., DO CHOI, Y., KIM, M., REUZEAU, C. & KIM, J.-K. 2010. Root-specific expression of OsNAC10 improves drought tolerance and grain yield in rice under field drought conditions. *Plant Physiology*, 153, 185-197.

JI, H., GHEYSEN, G., ULLAH, C., VERBEEK, R., SHANG, C., DE VLEESSCHAUWER, D., HÖFTE, M. & KYNDT, T. 2015. The role of thionins in rice defence against root pathogens. *Molecular Plant Pathology*, 16, 870-881.

JOO, J., LEE, Y. H., KIM, Y.-K., NAHM, B. H. & SONG, S. I. 2013. Abiotic stress responsive rice ASR1 and ASR3 exhibit different tissue-dependent sugar and hormone-sensitivities. *Molecules and cells*, 35, 421-435.

JOSHI, R., WANI, S. H., SINGH, B., BOHRA, A., DAR, Z. A., LONE, A. A., PAREEK, A. & SINGLA-PAREEK, S. L. 2016. Transcription factors and plants response to drought stress: current understanding and future directions. *Frontiers in Plant Science*, 7, 1029.

JUDD, L. A., JACKSON, B. E. & FONTENO, W. C. 2015. Advancements in root growth measurement technologies and observation capabilities for container-grown plants. *Plants*, 4, 369-392.

JURY, W. A. & VAUX, H. 2005. The role of science in solving the world's emerging water problems. *Proceedings of the National Academy of Sciences USA*, 102, 15715-15720.

KANG, Y., KHAN, S. & MA, X. 2009. Climate change impacts on crop yield, crop water productivity and food security–A review. *Progress in natural Science*, 19, 1665-1674.

KANNO, Y., OIKAWA, T., CHIBA, Y., ISHIMARU, Y., SHIMIZU, T., SANO, N., KOSHIBA, T., KAMIYA, Y., UEDA, M. & SEO, M. 2016. AtSWEET13 and AtSWEET14 regulate gibberellin-mediated physiological processes. *Nature communications*, 7, 13245.

KANO-NAKATA, M., INUKAI, Y., WADE, L. J., SIOPONGCO, J. D. C. & YAMAUCHI, A. 2011. Root development, water uptake, and shoot dry matter production under water deficit conditions in two CSSLs of rice: Functional roles of root plasticity. *Plant production science*, 14, 307-317.

KAUR, R., SINGH, K., DEOL, J., DASS, A. & CHOUDHARY, A. K. 2015. Possibilities of Improving Performance of Direct Seeded Rice Using Plant Growth Regulators: A Review. *Proceedings of the National Academy of Sciences*, *India Section B: Biological Sciences*, 85, 909-922.

KELL, D. B. 2011. Breeding crop plants with deep roots: their role in sustainable carbon, nutrient and water sequestration. *Annals of Botany*, 108, 407-418.

KHADEM, S. A., GALAVI, M., RAMRODI, M., MOUSAVI, S. R., ROUSTA, M. J. & REZVANI-MOGHADAM, P. 2010. Effect of animal manure and superabsorbent polymer on corn leaf relative water content, cell membrane stability and leaf chlorophyll content under dry condition. *Australian Journal of Crop Science*, 4, 642.

KIM, S., SON, T., PARK, S., LEE, I., LEE, B., KIM, H. & LEE, S. 2006. Influences of gibberellin and auxin on endogenous plant hormone and starch mobilization during rice seed germination under salt stress. *Journal of Environmental Biology*, 27, 181.

KIRKHAM, M. B. 2014. Field Capacity, Wilting Point, Available Water, and the Nonlimiting Water Range. In *Principles of soil and plant water relations*, *Second edition,* Burlington, MA: Elsevier Academic Press, USA, pp 153-170. doi.org/10.1016/C2013-0-12871-1.

KISHOR, K., POLAVARAPU, B., HIMA KUMARI, P., SUNITA, M. & SREENIVASULU, N. 2015. Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. *Frontiers in Plant Science*, 6, 544.

KITANAGA, Y., JIAN, C., HASEGAWA, M., YAZAKI, J., KISHIMOTO, N., KIKUCHI, S., NAKAMURA, H., ICHIKAWA, H., ASAMI, T. & YOSHIDA, S. 2006. Sequential regulation of gibberellin, brassinosteroid, and jasmonic acid biosynthesis occurs in rice coleoptiles to control the transcript levels of anti-microbial thionin genes. *Bioscience, Biotechnology, and Biochemistry*, 70, 2410-2419.

KRUGMAN, T., PELEG, Z., QUANSAH, L., CHAGUÉ, V., KOROL, A. B., NEVO, E., SARANGA, Y., FAIT, A., CHALHOUB, B. & FAHIMA, T. 2011. Alteration in expression of hormone-related genes in wild emmer wheat roots associated with drought adaptation mechanisms. *Functional & Integrative Genomics*, 11, 565-583.

KUCERA, B., COHN, M. A. & LEUBNER-METZGER, G. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Science Research*, 15, 281-307.

KUDOYAROVA, G., VESELOVA, S., HARTUNG, W., FARHUTDINOV, R., VESELOV, D. & SHARIPOVA, G. 2011. Involvement of root ABA and hydraulic conductivity in the control of water relations in wheat plants exposed to increased evaporative demand. *Planta*, 233, 87-94.

LAMBERS, H., CHAPIN III, F. S. & PONS, T. L. 2008. Photosynthesis. *Plant Physiological Ecology*. Springer.

LAMICHHANEA, J. R., MESSEANB, A. & RICCIC, P. 2019. Research and innovation priorities as defined by the Ecophyto plan to address current crop protection transformation challenges in France. *Advances in Agronomy*, 154, 81.

LARIO, L. D., RAMIREZ-PARRA, E., GUTIERREZ, C., SPAMPINATO, C. P. & CASATI, P. 2013. ANTI-SILENCING FUNCTION1 proteins are involved in ultraviolet-induced DNA damage repair and are cell cycle regulated by E2F transcription factors in Arabidopsis. *Plant Physiology*, 162, 1164-1177.

LAVENUS, J., GOH, T., ROBERTS, I., GUYOMARC'H, S., LUCAS, M., DE SMET, I., FUKAKI, H., BEECKMAN, T., BENNETT, M. & LAPLAZE, L. 2013. Lateral root development in Arabidopsis: fifty shades of auxin. *Trends in Plant Science*, 18, 450-458.

LEAH, R., KIGEL, J., SVENDSEN, I. & MUNDY, J. 1995. Biochemical and molecular characterization of a barley seed β-glucosidase. *Journal of Biological Chemistry*, 270, 15789-15797.

LENKA, S. K., KATIYAR, A., CHINNUSAMY, V. & BANSAL, K. C. 2011. Comparative analysis of drought-responsive transcriptome in Indica rice genotypes with contrasting drought tolerance. *Plant Biotechnology Journal*, 9, 315-327.

LEVCHENKO, V., KONRAD, K. R., DIETRICH, P., ROELFSEMA, M. R. G. & HEDRICH, R. 2005. Cytosolic abscisic acid activates guard cell anion channels without preceding Ca^{2+} signals. *Proceedings of the National Academy of Sciences, USA*, 102, 4203-4208.

LI, X., GUO, Z., LV, Y., CEN, X., DING, X., WU, H., LI, X., HUANG, J. & XIONG, L. 2017. Genetic control of the root system in rice under normal and drought stress conditions by genome-wide association study. *PLoS Genetics*, 13, e1006889.

LI, X., WANG, X., HE, K., MA, Y., SU, N., HE, H., STOLC, V., TONGPRASIT, W., JIN, W. & JIANG, J. 2008. High-resolution mapping of epigenetic modifications of the rice genome uncovers interplay between DNA methylation, histone methylation, and gene expression. *The Plant Cell*, 20, 259-276.

LI, Y., LI, P., WANG, Y., DONG, R., YU, H. & HOU, B. 2014. Genome-wide identification and phylogenetic analysis of Family-1 UDP glycosyltransferases in maize (*Zea mays* L.*). Planta*, 239, 1265-1279.

LIN, M. S. 1993. Changes in genetic diversity in the Japonica rice region of Taiwan. *Botanical Bulletin of Academia Sinica*, 34, 307-312.

LISAR, S. Y., RAHMAN, I. M., HOSSAIN, M. M. & MOTAFAKKERAZAD, R. 2012. Water stress in plants: Causes, effects and responses. In Ismail M. d., Rahman, M., Hasegawa, H. (eds.) *Water stress*. Open Access Publisher, http://www.intechopen.com/books/water-stress/water-stress-inplantscauses-effects-and-responses

LIU, J., LIAO, D., OANE, R., ESTENOR, L., YANG, X., LI, Z. & BENNETT, J. 2006. Genetic variation in the sensitivity of anther dehiscence to drought stress in rice. *Field Crops Research*, 97, 87-100.

LIU, X., ZHANG, H., ZHAO, Y., FENG, Z., LI, Q., YANG, H.-Q., LUAN, S., LI, J. & HE, Z.-H. 2013. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in Arabidopsis. *Proceedings of the National Academy of Sciences USA*, 110, 15485-15490.

LIU, Y., DING, Y., WANG, Q., MENG, D. & WANG, S. 2011. Effects of nitrogen and 6-benzylaminopurine on rice tiller bud growth and changes in endogenous hormones and nitrogen. *Crop Science*, 51, 786-792.

LJUNG, K., BHALERAO, R. P. & SANDBERG, G. 2001. Sites and homeostatic control of auxin biosynthesis in Arabidopsis during vegetative growth. *The Plant Journal*, 28, 465-474.

LJUNG, K., HULL, A. K., KOWALCZYK, M., MARCHANT, A., CELENZA, J., COHEN, J. D. & SANDBERG, G. 2002. Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in Arabidopsis thaliana. *Plant Molecular Biology*, 49, 249-272.

LOBELL, D. B. & GOURDJI, S. M. 2012. The influence of climate change on global crop productivity. *Plant Physiology*, 160, 1686-1697.

LOBET, G., PAGÈS, L. & DRAYE, X. 2011. A novel image-analysis toolbox enabling quantitative analysis of root system architecture. *Plant Physiology*, 157, 29-39.

LU, Y. C., YANG, S. N., ZHANG, J. J., ZHANG, J. J., TAN, L. R. & YANG, H. 2013. A collection of glycosyltransferases from rice (*Oryza sativa*) exposed to atrazine. *Gene*, 531, 243-252.

LUTTS, S., BENINCASA, P., WOJTYLA, L., KUBALA, S., PACE, R., LECHOWSKA, K., QUINET, M. & GARNCZARSKA, M. 2016. Seed priming: new comprehensive approaches for an old empirical technique. In: Susana Araujo, S., Balestrazzi, A. (Eds.) New challenges in seed biology-Basic and translational research driving seed technology. *InTechOpen*, Croatia.

LV, S., YU, D., SUN, Q. & JIANG, J. 2018. Activation of gibberellin 20-oxidase 2 undermines auxin-dependent root and root hair growth in NaCl-stressed Arabidopsis seedlings. *Plant Growth Regulation*, 84, 225-236.

LYNCH, J. 1995. Root architecture and plant productivity. *Plant Physiology*, 109, 7-13.

LYNCH, J. P. 2007. Roots of the second green revolution. *Australian Journal of Botany*, 55, 493-512.

LYNCH, J. P. 2013. Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Annals of Botany*, 112, 347-357.

MA, H.-Y., ZHAO, D.-D., NING, Q.-R., WEI, J.-P., LI, Y., WANG, M.-M., LIU, X.-L., JIANG, C.-J. & LIANG, Z.-W. 2018. A Multi-year Beneficial Effect of Seed Priming with Gibberellic Acid-3 (GA 3) on Plant Growth and Production in a Perennial Grass, *Leymus chinensis*. *Scientific Reports*, 8, 13214.

MAI, C. D., PHUNG, N. T., TO, H. T., GONIN, M., HOANG, G. T., NGUYEN, K. L., DO, V. N., COURTOIS, B. & GANTET, P. 2014. Genes controlling root development in rice. *Rice*, 7, 30.

MALAMY, J. E. 2005. Intrinsic and environmental response pathways that regulate root system architecture. *Plant Cell and Environment*, 28, 67-77.

MANO, Y. & NEMOTO, K. 2012. The pathway of auxin biosynthesis in plants. *Journal of Experimental Botany*, 63, 2853-2872.

MARCHANT, A., BHALERAO, R., CASIMIRO, I., EKLÖF, J., CASERO, P. J., BENNETT, M. & SANDBERG, G. 2002. AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling. *The Plant Cell*, 14, 589-597.

MARSCHNER, H. 1995. *Mineral nutrition of higher plants, Second edition. Academic Press, London.*

MÄSER, P., LEONHARDT, N. & SCHROEDER, J. I. 2003. The clickable guard cell: electronically linked model of guard cell signal transduction pathways. *The Arabidopsis Book*, American Society of Plant Biologists, 6, e0114. http://doi.org/10.1199/tab.0114.

MENG, F., XIANG, D., ZHU, J., LI, Y. & MAO, C. 2019. Molecular mechanisms of root development in rice. *Rice*, 12, 1-10.

METZNER, R., EGGERT, A., VAN DUSSCHOTEN, D., PFLUGFELDER, D., GERTH, S., SCHURR, U., UHLMANN, N. & JAHNKE, S. 2015. Direct comparison of MRI and X-ray CT technologies for 3D imaging of root systems in soil: potential and challenges for root trait quantification. *Plant Methods*, 11, 17.

MOHANTY, B., KITAZUMI, A., CHEUNG, C. M., LAKSHMANAN, M., BENILDO, G., JANG, I.-C. & LEE, D.-Y. 2016. Identification of candidate network hubs involved in metabolic adjustments of rice under drought stress by integrating transcriptome data and genome-scale metabolic network. *Plant Science*, 242, 224-239.

MORANT, A. V., JØRGENSEN, K., JØRGENSEN, C., PAQUETTE, S. M., SÁNCHEZ-PÉREZ, R., MØLLER, B. L. & BAK, S. 2008. β-Glucosidases as detonators of plant chemical defense. *Phytochemistry*, 69, 1795-1813.

MORISON, J. I., BAKER, N. R., MULLINEAUX, P. M. & DAVIES, W. J. 2008. Improving water use in crop production. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 363, 639-658.

MÜLLER, D. & LEYSER, O. 2011. Auxin, cytokinin and the control of shoot branching. *Annals of Botany*, 107, 1203-1212.

NADA, R. M., ABO-HEGAZY, S. E., BUDRAN, E. G. & ABOGADALLAH, G. M. 2019. The interaction of genes controlling root traits is required for the developmental acquisition of deep and thick root traits and improving root architecture in response to low water or nitrogen content in rice (*Oryza sativa* L.) cultivars. *Plant Physiology and Biochemistry*, 141, 122-132.

NAKASHIMA, K., ITO, Y. & YAMAGUCHI-SHINOZAKI, K. 2009. Transcriptional regulatory networks in response to abiotic stresses in Arabidopsis and grasses. *Plant Physiology*, 149, 88-95.

NAKASHIMA, K., TRAN, L. S. P., VAN NGUYEN, D., FUJITA, M., MARUYAMA, K., TODAKA, D., ITO, Y., HAYASHI, N., SHINOZAKI, K. & YAMAGUCHI-SHINOZAKI, K. 2007. Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. *The Plant Journal*, 51, 617-630.

NELSON, G. C., ROSEGRANT, M. W., KOO, J., ROBERTSON, R., SULSER, T., ZHU, T., RINGLER, C., MSANGI, S., PALAZZO, A. & BATKA, M. 2009. *Climate change: Impact on agriculture and costs of adaptation*, International Food Policy Research Institute, Washington.

NGUYEN, H. T., BABU, R. C. & BLUM, A. 1997. Breeding for drought resistance in rice: physiology and molecular genetics considerations. *Crop Science*, 37, 1426-1434.

NUCCIO, M. L., PAUL, M., BATE, N. J., COHN, J. & CUTLER, S. R. 2018. Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement. *Plant Science*, 273, 110–119.

OGAWA, M., HANADA, A., YAMAUCHI, Y., KUWAHARA, A., KAMIYA, Y. & YAMAGUCHI, S. 2003. Gibberellin biosynthesis and response during Arabidopsis seed germination. *The Plant Cell*, 15, 1591-1604.

OPASSIRI, R., CAIRNS, J. R. K., AKIYAMA, T., WARA-ASWAPATI, O., SVASTI, J. & ESEN, A. 2003. Characterization of a rice β-glucosidase highly expressed in flower and germinating shoot. *Plant Science*, 165, 627-638.

ORCUTT, D. M. 2000. *The physiology of plants under stress: soil and biotic factors*, John Wiley & Sons, New York.

OSTROWSKI, M. & JAKUBOWSKA, A. 2014. UDP-glycosyltransferases of plant hormones. *Advances in Cell Biology*, 4, 43-60.

OVERVOORDE, P., FUKAKI, H. & BEECKMAN, T. 2010. Auxin control of root development. *Cold Spring Harbor Perspectives in Biology*, 2, a001537. doi: 10.1101/cshperspect. a001537

PACHECO-VILLALOBOS, D. & HARDTKE, C. S. 2012. Natural genetic variation of root system architecture from Arabidopsis to Brachypodium: towards adaptive value. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, 367, 1552-1558.

PANDEY, P., RAMEGOWDA, V. & SENTHIL-KUMAR, M. 2015. Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. *Frontiers in Plant Science*, 6, 723.

PARK, J., KIM, Y.-S., KIM, S.-G., JUNG, J.-H., WOO, J.-C. W. & PARK, C.-M. 2011. Integration of auxin and salt signals by a NAC transcription factor NTM2 during seed germination in Arabidopsis. *Plant Physiology*, 156, 537-549.

PEER, W. A., BLAKESLEE, J. J., YANG, H. & MURPHY, A. S. 2011. Seven Things We Think We Know about Auxin Transport. *Molecular Plant*, 4, 487-504.

PELEG, Z., REGUERA, M., TUMIMBANG, E., WALIA, H. & BLUMWALD, E. 2011. Cytokinin-mediated source/sink modifications improve drought tolerance and increase grain yield in rice under water-stress. *Plant Biotechnology Journal*, 9, 747-758.

PEREIRA, L. S., CORDERY, I. & IACOVIDES, I. 2009. Coping with water scarcity: *Addressing the challenges*, Springer Science & Business Media.

PEREIRA, L. S., OWEIS, T. & ZAIRI, A. 2002. Irrigation management under water scarcity. *Agricultural Water Management*, 57, 175-206.

PÉRET, B., DE RYBEL, B., CASIMIRO, I., BENKOVÁ, E., SWARUP, R., LAPLAZE, L., BEECKMAN, T. & BENNETT, M. J. 2009. Arabidopsis lateral root development: an emerging story. *Trends in Plant Science*, 14, 399-408.

PIAO, S., CIAIS, P., HUANG, Y., SHEN, Z., PENG, S., LI, J., ZHOU, L., LIU, H., MA, Y. & DING, Y. 2010. The impacts of climate change on water resources and agriculture in China. *Nature*, 467, 43.

PILET, P. 2002. Root growth and gravireaction: A critical study of hormone and regulator implications. In *Plant Roots The Hidden Half*, 3rd. Edition, Waisel, Y., Eshel, A., Beeckman, T. and Kafkafi, U. (ed). pp 489-504. New York.

PINHEIRO, C. & CHAVES, M. 2010. Photosynthesis and drought: can we make metabolic connections from available data? *Journal of Experimental Botany*, 62, 869-882.

PRASAD, P., BOOTE, K., ALLEN JR, L., SHEEHY, J. & THOMAS, J. 2006a. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*, 95, 398-411.

PRASAD, P., BOOTE, K., ALLEN, L., SHEEHY, J. & THOMAS, J. 2006b. Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Research*, 95, 398-411.

PRICE, A. H., CAIRNS, J. E., HORTON, P., JONES, H. G. & GRIFFITHS, H. 2002. Linking drought-resistance mechanisms to drought avoidance in upland rice using a QTL approach: progress and new opportunities to integrate stomatal and mesophyll responses. *Journal of Experimental Botany*, 53, 989-1004.

PUCKRIDGE, D. & O'TOOLE, J. 1980. Dry matter and grain production of rice, using a line source sprinkler in drought studies. *Field Crops Research*, 3, 303-319.

QIN, J., HU, F., ZHANG, B., WEI, Z. & LI, H. 2006. Role of straw mulching in non-continuously flooded rice cultivation. *Agricultural Water Management*, 83, 252-260.

RABBANI, M. A., MARUYAMA, K., ABE, H., KHAN, M. A., KATSURA, K., ITO, Y., YOSHIWARA, K., SEKI, M., SHINOZAKI, K. & YAMAGUCHI-SHINOZAKI, K. 2003. Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiology*, 133, 1755-1767.

RAJJOU, L., DUVAL, M., GALLARDO, K., CATUSSE, J., BALLY, J., JOB, C. & JOB, D. 2012. Seed germination and vigour. *Annual Review of Plant Biology*, 63, 507-533.

RAMANATHAN, V. & FENG, Y. 2009. Air pollution, greenhouse gases and climate change: Global and regional perspectives. *Atmospheric Environment*, 43, 37-50.

RAO, I. M., MILES, J. W., BEEBE, S. E. & HORST, W. J. 2016. Root adaptations to soils with low fertility and aluminium toxicity. *Annals of Botany*, 118, 593-605.

RAY, D. K., GERBER, J. S., MACDONALD, G. K. & WEST, P. C. 2015. Climate variation explains a third of global crop yield variability. *Nature Communications*, 6, 5989.

REGNAULT, T., DAVIÈRE, J.-M. & ACHARD, P. 2016. Long-distance transport of endogenous gibberellins in Arabidopsis. *Plant Signaling & Behavior*, 11, e1110661. https://doi.org/10.1080/15592324.2015.1110661.

REHMAN, H. 2011. Seed priming with CaCl2 improves the stand establishment, yield and quality attributes in direct seeded rice (*Oryza sativa* L.). *International Journal of Agriculture and Biology*, 13, 786–790.

REHMAN, H. U., BASRA, S., RADY, M. M., GHONEIM, A. M. & WANG, Q. 2017. Moringa leaf extract improves wheat growth and productivity by affecting senescence and source-sink relationship. *International Journal of Agriculture & Biology*, 19, 479–484.

REHMAN, H. U., BASRA, S. M. A. & FAROOQ, M. 2011. Field appraisal of seed priming to improve the growth, yield, and quality of direct seeded rice. *Turkish Journal of Agriculture and Forestry*, 35, 357-365.

REYNOLDS, M. P. 2010. *Climate change and crop production*, CABI.

RIBAUT, J.-M. & RAGOT, M. 2006. Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. *Journal of Experimental Botany*, 58, 351-360.

RICH, S. M. & WATT, M. 2013. Soil conditions and cereal root system architecture: review and considerations for linking Darwin and Weaver. *Journal of Experimental Botany*, 64, 1193-1208.

RINGLER, C., BHADURI, A. & LAWFORD, R. 2013. The nexus across water, energy, land and food (WELF): potential for improved resource use efficiency? *Current Opinion in Environmental Sustainability*, 5, 617-624.

ROOPENDRA, K., SHARMA, A., CHANDRA, A. & SAXENA, S. 2018. Gibberellin-induced perturbation of source–sink communication promotes sucrose accumulation in sugarcane. *3 Biotech*, 8, 418. https://doi.org/10.1007/s13205-018-1429-2.

RUYTER-SPIRA, C., KOHLEN, W., CHARNIKHOVA, T., VAN ZEIJL, A., VAN BEZOUWEN, L., DE RUIJTER, N., CARDOSO, C., LOPEZ-RAEZ, J. A., MATUSOVA, R. & BOURS, R. 2011. Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? *Plant Physiology*, 155, 721-734.

SAITO, S., OKAMOTO, M., SHINODA, S., KUSHIRO, T., KOSHIBA, T., KAMIYA, Y., HIRAI, N., TODOROKI, Y., SAKATA, K. & NAMBARA, E. 2006. A plant growth retardant, uniconazole, is a potent inhibitor of ABA catabolism in Arabidopsis. *Bioscience, Biotechnology, and Biochemistry*, 70, 1731-1739.

SAKAMOTO, T., MIURA, K., ITOH, H., TATSUMI, T., UEGUCHI-TANAKA, M., ISHIYAMA, K., KOBAYASHI, M., AGRAWAL, G. K., TAKEDA, S. & ABE, K. 2004. An overview of gibberellin metabolism enzyme genes and their related mutants in rice. *Plant Physiology*, 134, 1642-1653.

SAKAMOTO, T., MORINAKA, Y., ISHIYAMA, K., KOBAYASHI, M., ITOH, H., KAYANO, T., IWAHORI, S., MATSUOKA, M. & TANAKA, H. 2003. Genetic manipulation of gibberellin metabolism in transgenic rice. *Nature Biotechnology*, 21, 909.

SAKURAI, J., ISHIKAWA, F., YAMAGUCHI, T., UEMURA, M. & MAESHIMA, M. 2005. Identification of 33 rice aquaporin genes and analysis of their expression and function. *Plant and Cell Physiology*, 46, 1568-1577.

SALEHIN, M., BAGCHI, R. & ESTELLE, M. 2015. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *The Plant Cell*, 27, 9-19.

SANTINO, C. G., STANFORD, G. L. & CONNER, T. W. 1997. Developmental and transgenic analysis of two tomato fruit enhanced genes. *Plant molecular biology*, 33, 405-416.

SANTNER, A., CALDERON-VILLALOBOS, L. I. A. & ESTELLE, M. 2009. Plant hormones are versatile chemical regulators of plant growth. *Nature Chemical Biology*, 5, 301.

SAYOLS, S., SCHERZINGER, D. & KLEIN, H. 2016. dupRadar: a Bioconductor package for the assessment of PCR artifacts in RNA-Seq data. *BMC bioinformatics*, 17, 428.

SCHACHTMAN, D. P. & GOODGER, J. Q. D. 2008. Chemical root to shoot signaling under drought. *Trends in Plant Science*, 13, 281-287.

SCHULZE, E.-D. & KÜPPERS, M. 1979. Short-term and long-term effects of plant water deficits on stomatal response to humidity in *Corylus avellana* L. *Planta*, 146, 319-326.

SCOTT, T. K. 1972. Auxins and roots. *Annual Review of Plant Physiology*, 23, 235-258.

SECK, P. A., DIAGNE, A., MOHANTY, S. & WOPEREIS, M. C. 2012. Crops that feed the world 7: rice. *Food Security*, 4, 7-24.

SELOTE, D. S. & KHANNA-CHOPRA, R. 2004. Drought-induced spikelet sterility is associated with an inefficient antioxidant defence in rice panicles. *Physiologia Plantarum*, 121, 462-471.

SERRAJ, R., MCNALLY, K. L., SLAMET-LOEDIN, I., KOHLI, A., HAEFELE, S. M., ATLIN, G. & KUMAR, A. 2011. Drought resistance improvement in rice: an integrated genetic and resource management strategy. *Plant Production Science*, 14, 1-14.

SHAH, S., HOUBORG, R. & MCCABE, M. 2017. Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy*, 7, 61.

SHANI, E., WEINSTAIN, R., ZHANG, Y., CASTILLEJO, C., KAISERLI, E., CHORY, J., TSIEN, R. Y. & ESTELLE, M. 2013. Gibberellins accumulate in the elongating endodermal cells of Arabidopsis root. *Proceedings of the National Academy of Sciences USA*, 110, 4834-4839.

SHARP, R. E., POROYKO, V., HEJLEK, L. G., SPOLLEN, W. G., SPRINGER, G. K., BOHNERT, H. J. & NGUYEN, H. T. 2004. Root growth maintenance during water deficits: physiology to functional genomics. *Journal of Experimental Botany*, 55, 2343-2351.

SHETEIWY, M. S., GONG, D., GAO, Y., PAN, R., HU, J. & GUAN, Y. 2018. Priming with methyl jasmonate alleviates polyethylene glycol-induced osmotic stress in rice seeds by regulating the seed metabolic profile. *Environmental and Experimental Botany*, 153, 236-248.

SHIMADA, A., UEGUCHI-TANAKA, M., NAKATSU, T., NAKAJIMA, M., NAOE, Y., OHMIYA, H., KATO, H. & MATSUOKA, M. 2008. Structural basis for gibberellin recognition by its receptor GID1. *Nature*, 456, 520.

SHINOZAKI, K. & YAMAGUCHI-SHINOZAKI, K. 2007. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany*, 58, 221-227.

SHINOZAKI, K., YAMAGUCHI-SHINOZAKI, K. & SEKI, M. 2003. Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology*, 6, 410-417.

SIDIRAS, N. & KARSIOTI, S. 1996. Effects of seed size and seed substances of lupins on seedling emergence and root system development in relation to sowing depth, soil water and gibberellin. *Journal of Agronomy and Crop Science*, 177, 73-83.

SKIRYCZ, A. & INZÉ, D. 2010. More from less: plant growth under limited water. *Current Opinion in Biotechnology*, 21, 197-203.

SPIELMEYER, W., ELLIS, M. H. & CHANDLER, P. M. 2002. Semidwarf (sd-1),"green revolution" rice, contains a defective gibberellin 20-oxidase gene. *Proceedings of the National Academy of Sciences USA*, 99, 9043-9048.

STEIN, H., HONIG, A., MILLER, G., ERSTER, O., EILENBERG, H., CSONKA, L. N., SZABADOS, L., KONCZ, C. & ZILBERSTEIN, A. 2011. Elevation of free proline and proline-rich protein levels by simultaneous manipulations of proline biosynthesis and degradation in plants. *Plant Science*, 181, 140-150.

SUN, T.-P. 2010. Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development. *Plant Physiology*, 154, 567-570.

SURALTA, R. R. & YAMAUCHI, A. 2008. Root growth, aerenchyma development, and oxygen transport in rice genotypes subjected to drought and waterlogging. *Environmental and Experimental Botany*, 64, 75-82.

SWAMY, B. M. & KUMAR, A. 2012. Sustainable rice yield in water-short drought-prone environments: Conventional and molecular approaches, Citeseer.

Swamy BPM, Kumar A: Sustainable rice yield in water short drought prone environments: conventional and molecular approaches. In Lee T. L. (ed) *Irrigation systems and practices in challenging environments*. Croatia: InTech, 149-168.

TABUR, S. & ÖNEY, S. 2012. Comparison of cytogenetic antagonism between abscisic acid and plant growth regulators. *Pakistan Journal of Botany*, 44, 1581-1586.

TAIZ, L. & ZEIGER, E. 2006. Abscisic acid: a seed maturation and stress signal. In Plant *Physiology* (4th edition) Chap, 23.

TAKAHASHI, H., SAIKA, H., MATSUMURA, H., NAGAMURA, Y., TSUTSUMI, N., NISHIZAWA, N. K. & NAKAZONO, M. 2011. Cell division and cell elongation in the coleoptile of rice alcohol dehydrogenase 1-deficient mutant are reduced under complete submergence. *Annals of Botany*, 108, 253-261.

TANIMOTO, E. 1987. Gibberellin-dependent root elongation in *Lactuca sativa*: recovery from growth retardant-suppressed elongation with thickening by low concentration of GA3. *Plant and Cell Physiology*, 28, 963-973.

TANIMOTO, E. 1988. Gibberellin regulation of root growth with change in galactose content of cell walls in Pisum sativum. *Plant and Cell Physiology*, 29, 269-280.

TANIMOTO, E. 1994. Interaction of gibberellin A3 and ancymidol in the growth and cell-wall extensibility of dwarf pea roots. *Plant and Cell Physiology*, 35, 1019-1028.

TANIMOTO, E. 2005. Regulation of root growth by plant hormones—roles for auxin and gibberellin. *Critical Reviews in Plant Sciences*, 24, 249-265.

TARDIEU, F., PARENT, B. & SIMONNEAU, T. 2010. Control of leaf growth by abscisic acid: hydraulic or non-hydraulic processes? *Plant, Cell & Environment*, 33, 636-647.

TAYLOR, R. G., SCANLON, B., DÖLL, P., RODELL, M., VAN BEEK, R., WADA, Y., LONGUEVERGNE, L., LEBLANC, M., FAMIGLIETTI, J. S. & EDMUNDS, M. 2013. Ground water and climate change. *Nature Climate Change*, 3, 322.

THOMPSON, A. J., MULHOLLAND, B. J., JACKSON, A. C., MCKEE, J. M., HILTON, H. W., SYMONDS, R. C., SONNEVELD, T., BURBIDGE, A., STEVENSON, P. & TAYLOR, I. B. 2007. Regulation and manipulation of ABA biosynthesis in roots. *Plant, Cell & Environment*, 30, 67-78.

TIAN, H., JIA, Y., NIU, T., YU, Q. & DING, Z. 2014. The key players of the primary root growth and development also function in lateral roots in Arabidopsis. *Plant Cell Reports*, 33, 745-753.

TIERNEY, M. L., WIECHERT, J. & PLUYMERS, D. 1988. Analysis of the expression of extensin and p33-related cell wall proteins in carrot and soybean. *Molecular and General Genetics*, 211, 393-399.

TRAN, D. 2004. Rice and narrowing the yield gaps. Food and Agriculture Organization (FAO).

TRAN, L.-S. P., SHINOZAKI, K. & YAMAGUCHI-SHINOZAKI, K. 2010. Role of cytokinin responsive two-component system in ABA and osmotic stress signalings. *Plant Signaling & Behavior*, 5, 148-150.

TRON, S., BODNER, G., LAIO, F., RIDOLFI, L. & LEITNER, D. 2015. Can diversity in root architecture explain plant water use efficiency? A modeling study. *Ecological Modelling*, 312, 200-210.

TSUBO, M., BASNAYAKE, J., FUKAI, S., SIHATHEP, V., SIYAVONG, P., SIPASEUTH & CHANPHENGSAY, M. 2006. Toposequential effects on water balance and productivity in rainfed lowland rice ecosystem in Southern Laos. *Field Crops Research*, 97, 209-220.

UBEDA-TOMAS, S., FEDERICI, F., CASIMIRO, I., BEEMSTER, G. T. S., BHALERAO, R., SWARUP, R., DOERNER, P., HASELOFF, J. & BENNETT, M. J. 2009. Gibberellin signaling in the endodermis controls Arabidopsis root meristem size. *Current Biology*, 19, 1194-1199.

UEGUCHI-TANAKA, M., NAKAJIMA, M., KATOH, E., OHMIYA, H., ASANO, K., SAJI, S., HONGYU, X., ASHIKARI, M., KITANO, H. & YAMAGUCHI, I. 2007. Molecular interactions of a soluble gibberellin receptor, GID1, with a rice DELLA protein, SLR1, and gibberellin. *The Plant Cell*, 19, 2140-2155.

UENO, K. & MIYOSHI, K. 2005. Difference of optimum germination temperature of seeds of intact and dehusked japonica rice during seed development. *Euphytica*, 143, 271-275.

UGA, Y., KITOMI, Y., YAMAMOTO, E., KANNO, N., KAWAI, S., MIZUBAYASHI, T. & FUKUOKA, S. 2015. A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of DEEPER ROOTING 1. *Rice*, 8, 8.

UGA, Y., SUGIMOTO, K., OGAWA, S., RANE, J., ISHITANI, M., HARA, N., KITOMI, Y., INUKAI, Y., ONO, K. & KANNO, N. 2013. Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. *Nature Genetics*, 45, 1097-1102.

ULFAT, A., MAJID, S. A. & HAMEED, A. 2017. Hormonal seed priming improves wheat (*Triticum aestivum* L.) field performance under drought and non-stress conditions. *Pakistan Journal of Botany*, 49, 1239-1253.

UMEHARA, M., HANADA, A., MAGOME, H., TAKEDA-KAMIYA, N. & YAMAGUCHI, S. 2010. Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. *Plant and Cell Physiology*, 51, 1118-1126.

VADEZ, V. 2014. Root hydraulics: the forgotten side of roots in drought adaptation. *Field Crops Research*, 165, 15-24.

VADEZ, V., KHOLOVA, J., ZAMAN-ALLAH, M. & BELKO, N. 2013. Water: the most important 'molecular'component of water stress tolerance research. *Functional Plant Biology*, 40, 1310-1322.

VAN HA, C., LEYVA-GONZÁLEZ, M. A., OSAKABE, Y., TRAN, U. T., NISHIYAMA, R., WATANABE, Y., TANAKA, M., SEKI, M., YAMAGUCHI, S. & VAN DONG, N. 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. *Proceedings of the National Academy of Sciences USA*, 111, 851-856.

VANNESTE, S. & FRIML, J. 2009. Auxin: a trigger for change in plant development. *Cell*, 136, 1005-1016.

VERMA, V., RAVINDRAN, P. & KUMAR, P. P. 2016. Plant hormone-mediated regulation of stress responses. *BMC Plant Biology*, 16, 86.

VISHAL, B. & KUMAR, P. P. 2018. Regulation of seed germination and abiotic stresses by gibberellins and abscisic acid. *Frontiers in Plant Science,* 9*, 838.*

WANG, J., LU, K., NIE, H. P., ZENG, Q. S., WU, B. W., QIAN, J. J. & FANG, Z. M. 2018. Rice nitrate transporter OsNPF7.2 positively regulates tiller number and grain yield. *Rice*, 11.

WANI, S. H., KUMAR, V., SHRIRAM, V. & SAH, S. K. 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal*, 4, 162-176.

World Water Assessment Programme. 2009. The United Nations World Water Development Report 3: Water in a Changing World. Paris: UNESCO, and London: Earthscan.

WEITBRECHT, K., MÜLLER, K. & LEUBNER-METZGER, G. 2011. First off the mark: early seed germination. *Journal of Experimental Botany*, 62, 3289-3309.

WERNER, T., MOTYKA, V., LAUCOU, V., SMETS, R., VAN ONCKELEN, H. & SCHMÜLLING, T. 2003. Cytokinin-deficient transgenic Arabidopsis plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *The Plant Cell*, 15, 2532-2550.

WERNER, T., MOTYKA, V., STRNAD, M. & SCHMÜLLING, T. 2001. Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences USA*, 98, 10487-10492.

WERNER, T., NEHNEVAJOVA, E., KÖLLMER, I., NOVÁK, O., STRNAD, M., KRÄMER, U. & SCHMÜLLING, T. 2010. Root-specific reduction of cytokinin causes enhanced root growth, drought tolerance, and leaf mineral enrichment in Arabidopsis and tobacco. *The Plant Cell*, 22, 3905-3920.

WERNER, T. & SCHMÜLLING, T. 2009. Cytokinin action in plant development. *Current Opinion in Plant Biology*, 12, 527-538.

WILKINS, M. B. 1969. Circadian rhythms in plants. In: *Physiology of plant growth and development*, Wilkins, M. B. ed. McGraw- Hill, London. pp. 647-671.

WILKINSON, S. & DAVIES, W. J. 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant Cell and Environment*, 25, 195-210.

WOOL, R. & SUN, X. S. 2011. Plant materials formation and growth. In Bio-based polymers and composites, Elsevier. Academic press, UK.

WOPEREIS, M., KROPFF, M., MALIGAYA, A. & TUONG, T. 1996. Drought-stress responses of two lowland rice cultivars to soil water status. *Field Crops Research*, 46, 21-39.

XU, D.-Q., HUANG, J., GUO, S.-Q., YANG, X., BAO, Y.-M., TANG, H.-J. & ZHANG, H.-S. 2008. Overexpression of a TFIIIA-type zinc finger protein gene ZFP252 enhances drought and salt tolerance in rice (*Oryza sativa* L.). *FEBS Letters*, 582, 1037-1043.

XU, J., LIAO, L., TAN, J. & SHAO, X. 2013. Ammonia volatilization in gemmiparous and early seedling stages from direct seeding rice fields with different nitrogen management strategies: A pots experiment. *Soil and Tillage Research*, 126, 169-176.

XU, M., ZHU, L., SHOU, H. & WU, P. 2005. A PIN1 family gene, OsPIN1, involved in auxin-dependent adventitious root emergence and tillering in rice. *Plant and Cell Physiology*, 46, 1674-1681.

YAMAGUCHI-SHINOZAKI, K. & SHINOZAKI, K. 2005. Organization of cis-acting regulatory elements in osmotic-and cold-stress-responsive promoters. *Trends in Plant Science*, 10, 88-94.

YAMAMOTO, M. & YAMAMOTO, K. T. 1998. Differential effects of 1-naphthaleneacetic acid, indole-3-acetic acid and 2, 4-dichlorophenoxyacetic acid on the gravitropic response of roots in an auxin-resistant mutant of Arabidopsis, *aux1*. *Plant and Cell Physiology*, 39, 660-664.

YAMAMOTO, Y., KAMIYA, N., MORINAKA, Y., MATSUOKA, M. & SAZUKA, T. 2007. Auxin biosynthesis by the YUCCA genes in rice. *Plant Physiology*, 143, 1362-1371.

YAMAUCHI, Y., OGAWA, M., KUWAHARA, A., HANADA, A., KAMIYA, Y. & YAMAGUCHI, S. 2004. Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of Arabidopsis thaliana seeds. *The Plant Cell*, 16, 367-378.

YAN, C.-J., TIAN, Z.-X., FANG, Y.-W., YANG, Y.-C., LI, J., ZENG, S.-Y., GU, S.-L., XU, C.-W., TANG, S.-Z. & GU, M.-H. 2011. Genetic analysis of starch paste viscosity parameters in glutinous rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 122, 63-76.

YANG, J., ZHANG, J., WANG, Z., ZHU, Q. & LIU, L. 2002. Abscisic acid and cytokinins in the root exudates and leaves and their relationship to senescence and remobilization of carbon reserves in rice subjected to water stress during grain filling. *Planta*, 215, 645-652.

YANG, S., VANDERBELD, B., WAN, J. & HUANG, Y. 2010. Narrowing down the targets: towards successful genetic engineering of drought-tolerant crops. *Molecular Plant*, 3, 469-490.

YAXLEY, J. R., ROSS, J. J., SHERRIFF, L. J. & REID, J. B. 2001. Gibberellin biosynthesis mutations and root development in pea. *Plant Physiology*, 125, 627-633.

YE, N., JIA, L. & ZHANG, J. 2012. ABA signal in rice under stress conditions. *Rice*, 5, 1.

YU, P., WHITE, P. J. & LI, C. 2015. New insights to lateral rooting: Differential responses to heterogeneous nitrogen availability among maize root types. *Plant Signaling & Behavior*, 10, DOI: 10.1080/15592324.2015.1013795.

YUE, B., XUE, W., XIONG, L., YU, X., LUO, L., CUI, K., JIN, D., XING, Y. & ZHANG, Q. 2006. Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. *Genetics*, 172, 1213-1228.

ZAOCHANG, L., GOTO, Y. & NISHIYAMA, I. 2000. Effects of benzylaminopurine on shoot and root development and growth of rice (cv. north rose) grown hydroponically with different nitrogen forms. *Plant Production Science*, 3, 349-353.

ZAZIMALOVA, E. & NAPIER, R. 2003. Points of regulation for auxin action. *Plant Cell Reports*, 21, 625-634.

ZHA, M., IMRAN, M., WANG, Y., XU, J., DING, Y. & WANG, S. H. 2019. Transcriptome analysis revealed the interaction among strigolactones, auxin, and cytokinin in controlling the shoot branching of rice. Plant Cell Reports, 38, 279-293.

ZHANG, J., JIA, W., YANG, J. & ISMAIL, A. M. 2006. Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Research*, 97, 111-119.

ZHANG, S.-W., LI, C.-H., CAO, J., ZHANG, Y.-C., ZHANG, S.-Q., XIA, Y.-F., SUN, D.-Y. & SUN, Y. 2009. Altered architecture and enhanced drought tolerance in rice via the down-regulation of indole-3-acetic acid by TLD1/OsGH3. 13 activation. *Plant Physiology*, 151, 1889-1901.

ZHANG, Z., ZHANG, S., YANG, J. & ZHANG, J. 2008. Yield, grain quality and water use efficiency of rice under non-flooded mulching cultivation. *Field Crops Research*, 108, 71-81.

ZHANG, Z., ZHUO, X., YAN, X. & ZHANG, Q. 2018. Comparative genomic and transcriptomic analyses of family-1 udp glycosyltransferase in *Prunus mume*. *International Journal of Molecular Sciences*, 19, 3382.

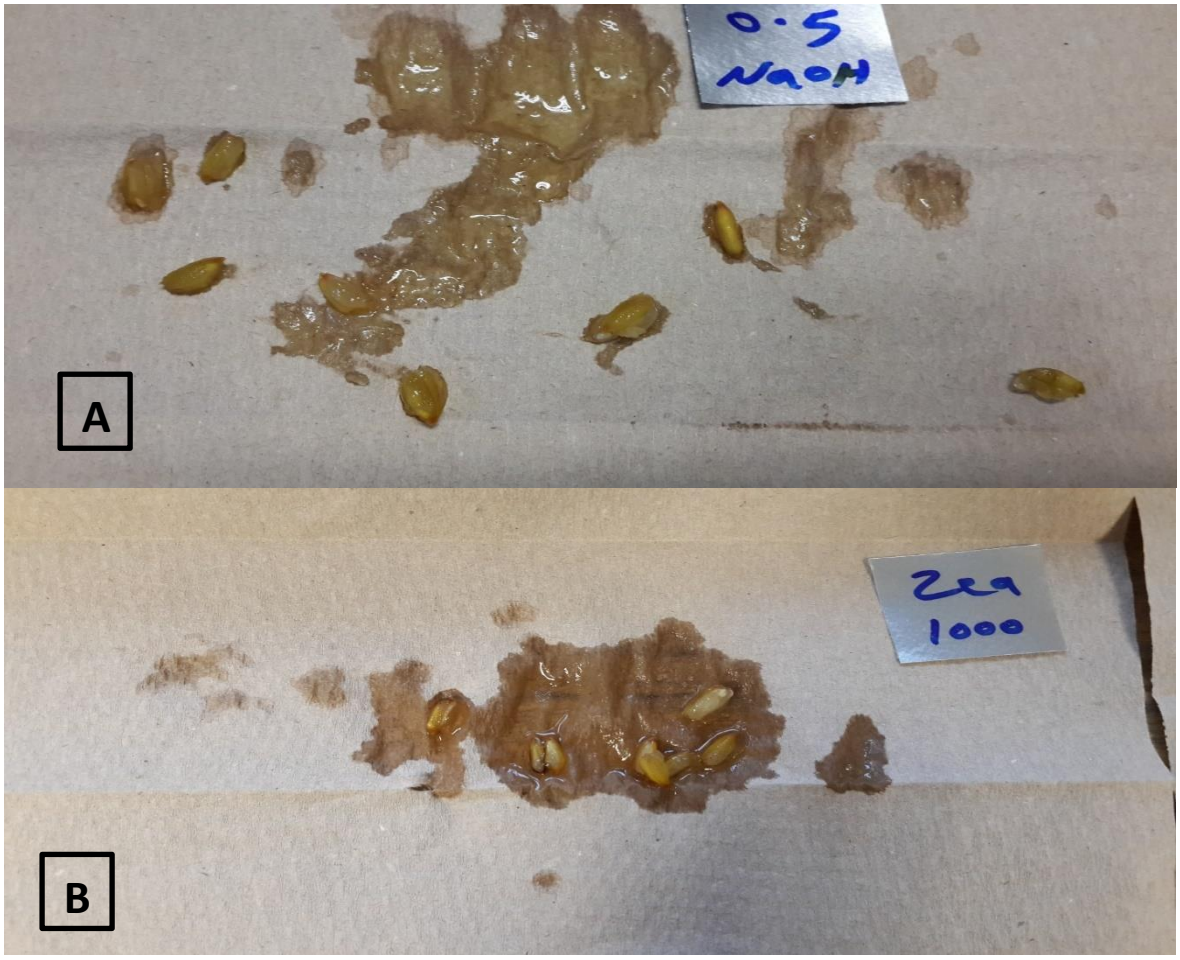
ZHAO, C., LIU, B., PIAO, S., WANG, X., LOBELL, D. B., HUANG, Y., HUANG, M., YAO, Y., BASSU, S. & CIAIS, P. 2017. Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences USA*, 114, 9326-9331.

ZHAO, H., MA, T., WANG, X., DENG, Y., MA, H., ZHANG, R. & ZHAO, J. 2015. *OsAUX1* controls lateral root initiation in rice (*Oryza sativa* L.). *Plant, Cell & Environment*, 38, 2208-2222.

ZHOU, L., LIU, Z., LIU, Y., KONG, D., LI, T., YU, S., MEI, H., XU, X., LIU, H. & CHEN, L. 2016. A novel gene *OsAHL1* improves both drought avoidance and drought tolerance in rice. *Scientific Reports*, 6, 30264.

ZHU, J., KAEPPLER, S. M. & LYNCH, J. P. 2005. Mapping of QTLs for lateral root branching and length in maize (*Zea mays* L.) under differential phosphorus supply. *Theoretical and Applied Genetics*, 111, 688-695.

Appendix 1



The Negative effect of soaking IR64 seed in 1000 µM zeatin on rice seed germination. (A) Seeds were soaked in 0.5 mM NaOH for 24 hrs and then placed on moist paper towels in an illuminated temperature-controlled incubator set to 34/11°C day/night. (B) Seeds were soaked in 1000 µM zeatin, which was dissolved in 1M NaOH, for 24 hrs and then placed on moist paper towels in an illuminated temperature-controlled incubator set to 34/11°C day/night. NaOH of 1 M solution was prepared by adding 40 g of NaOH to 1L distilled water.

Appendix 2

Lancrop Laboratories

Analysis Results (SOIL)

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Sample Ref NO 2

Date Received 17/05/2018

Sample No E202987/02

Crop RICE

Analysis	Result	Guideline	Interpretation	Comments
pH	7.5	6.5	High	High. An alkaline environment will reduce the availability of certain nutrients - particularly P, K, B, Co, Cu, Fe, Mn and Zn. An elevated pH will also impact on beneficial soil fungal populations and activity.
Phosphorus (ppm)	21	26	Slightly Low	**(Index 2)** Slightly low. Use appropriate quality phosphorus fertiliser.
Potassium (ppm)	96	241	Low	**(Index 1)** Low. Use appropriate quality potassium fertiliser.
Magnesium (ppm)	57	50	Normal	**(Index 2)** Adequate level.
Calcium (ppm)	564	1600	Very Low	Low priority on this crop. Other crops may be affected.
Sulphur (ppm)	11	10	Normal	Adequate level.
Manganese (ppm)	17	95	Very Low	Very low. Use appropriate quality manganese fertiliser.
Copper (ppm)	3.3	4.1	Slightly Low	Slightly low.
Boron (ppm)	0.68	0.50	Normal	Adequate level.
Zinc (ppm)	2.4	4.1	Low	Low. Use appropriate quality zinc fertiliser.
Molybdenum (ppm)	0.03	0.20	Very Low	Low priority on this crop. Other crops may be affected.
Iron (ppm)	444	50	Normal	Adequate level.
Sodium (ppm)	18	300	Very Low	No Problem.
C.E.C. (meq/100g)	3.6	15.0	Very Low	Cation Exchange Capacity indicates a very low nutrient holding ability - soil applied nutrients will be readily leached. Where possible foliar applied nutrients should be recommended.
Nitrogen Total (mg/kg)	239	1000	Very Low	Very low. Appropriate fertilisation essential.
Phosphorus Total (mg/kg)	80.1			

Additional Comments
Additional technical bulletins are available at www.lancrop.com

Please Note
Whilst every care is taken to ensure that the Results from Analysis are as accurate as possible, it is important to note that the analysis relates to the sample received by the laboratory, and is representative only of that sample. No warranty is given by the laboratory that the Results from Analysis relates to any part of a field or growing area not covered by the sample received. It is important to ensure that any soil, leaf, silage or fruitlet sample sent for analysis is representative of the area requiring analysis and that samples are obtained in accordance with established sampling techniques. A leaflet containing instructions on how to take soil, leaf, herbage, silage and fruit samples for analysis is available from the laboratory on request.

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Date Printed : 24/05/2018

Soil analysis for the soil experiment.

Appendix 3: REML outputs for statistical analyses in Chapter 3

Table 1. Statistical (REML) analysis for Figure 3.10 GA3 3000 µM primary root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	734.26	19	38.65	1252.0	<0.001
Concentration	25.59	1	25.59	1252.0	<0.001
Varieties. Concentration	104.19	19	5.48	1252.0	<0.001

Table 2. Statistical (REML) analysis for Figure 3.11 GA3 3000 µM lateral root count.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	405.69	19	21.35	1252.0	<0.001
Concentration	255.34	1	255.34	1252.0	<0.001
Varieties. Concentration	108.59	19	5.72	1252.0	<0.001

Table 3. Statistical (REML) analysis for Figure 3.12 GA3 3000 µM lateral root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	288.87	19	15.20	1259.0	<0.001
Concentration	330.19	1	330.19	1259.1	<0.001
Varieties. Concentration	114.46	19	6.02	1259.0	<0.001

Table 4. Statistical (REML) analysis for Figure 3.13 GA3 3000 µM total root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	496.79	19	26.15	1252.0	<0.001
Concentration	77.26	1	77.26	1252.0	<0.001
Varieties. Concentration	98.38	19	5.18	1252.0	<0.001

Table 5. Statistical (REML) analysis for Figure 3.14 GA3 3000 µM shoot length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	952.56	19	50.13	1269.0	<0.001
Concentration	883.11	1	883.11	1269.1	<0.001
Varieties. Concentration	157.34	19	8.28	1269.0	<0.001

Table 6. Statistical (REML) analysis for Figure 3.15 GA3 3000 µM aerial root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	865.48	19	45.55	1270.0	<0.001
Concentration	260.14	1	260.14	1270.0	<0.001
Varieties. Concentration	240.18	19	12.64	1270.0	<0.001

Table 7. Statistical (REML) analysis for Figure 3.16 GA3 1000 µM primary root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	559.44	19	29.44	1220.0	<0.001
Concentration	14.36	1	14.36	1220.0	<0.001
Varieties. Concentration	157.40	19	8.28	1220.0	<0.001

Table 8. Statistical (REML) analysis for Figure 3.17 GA3 1000 µM lateral root count.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	457.89	19	24.10	1220.0	<0.001
Concentration	123.84	1	123.84	1220.0	<0.001
Varieties. Concentration	90.56	19	4.77	1220.0	<0.001

Table 9. statistical (REML) analysis for figure 3.18 GA3 1000 µM lateral root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	307.60	19	16.19	1220.1	<0.001
Concentration	169.51	1	169.51	1220.0	<0.001
Varieties. Concentration	86.92	19	4.57	1220.0	<0.001

Table 10. Statistical (REML) analysis for Figure 3.19 GA3 1000 µM total root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	437.89	19	23.05	1220.0	<0.001
Concentration	42.33	1	42.33	1220.0	<0.001
Varieties. Concentration	124.70	19	6.56	1220.0	<0.001

Table 10. Statistical (REML) analysis for Figure 3.20 GA3 1000 µM shoot length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	1016.16	19	53.48	1237.0	<0.001
Concentration	635.16	1	635.16	1237.0	<0.001
Varieties. Concentration	93.04	19	4.90	1237.0	<0.001

Table 11. Statistical (REML) analysis for Figure 3.21 GA3 1000 µM aerial root number.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	463.81	19	24.41	1237.1	<0.001
Concentration	2.64	1	2.64	1237.0	0.105
Varieties. Concentration	60.33	19	3.18	1237.0	<0.001

Table 12. Statistical (REML) analysis for Figure 3.22 GA3 1000 µM aerial root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	802.29	19	42.23	1238.0	<0.001
Concentration	125.47	1	125.47	1238.0	<0.001
Varieties. Concentration	200.34	19	10.54	1238.0	<0.001

Table 13. Statistical (REML) analysis for Figure 3.23 NAA 250 µM primary root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	708.14	19	37.27	1233.0	<0.001
Concentration	0.91	1	0.91	1233.0	0.341
Varieties. Concentration	186.95	19	9.84	1233.0	<0.001

Table 14. Statistical (REML) analysis for Figure 3.24 NAA 250 µM lateral root count.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	550.72	19	28.99	1233.0	<0.001
Concentration	4.29	1	4.29	1233.0	0.038
Varieties. Concentration	46.18	19	2.43	1233.0	<0.001

Table 15. Statistical (REML) analysis for Figure 3.25 NAA 250 µM lateral root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	399.05	19	21.00	1234.0	<0.001
Concentration	0.03	1	0.03	1234.0	0.864
Varieties. Concentration	51.12	19	2.69	1234.0	<0.001

Table 16. Statistical (REML) analysis for Figure 3.26 NAA 250 µM total root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	540.11	19	28.43	1233.0	<0.001
Concentration	0.14	1	0.14	1233.0	0.713
Varieties. Concentration	105.97	19	5.58	1233.0	<0.001

Table 17. Statistical (REML) analysis for Figure 3.27 NAA 250 µM shoot length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	1172.65	19	61.72	1251.0	<0.001
Concentration	1.12	1	1.12	1251.0	0.289
Varieties. Concentration	51.69	19	2.72	1251.0	<0.001

Table 18. Statistical (REML) analysis for Figure 3.28 NAA 250 µM aerial root number.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	489.29	19	25.75	1251.0	<0.001
Concentration	2.54	1	2.54	1251.1	0.111
Varieties. Concentration	116.14	19	6.11	1251.0	<0.001

Table 19. Statistical (REML) analysis for Figure 3.29 NAA 250 µM aerial root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	944.33	19	49.70	1251.0	<0.001
Concentration	60.94	1	60.94	1251.0	<0.001
Varieties. Concentration	227.02	19	11.95	1251.0	<0.001

Table 20. Statistical (REML) analysis for Figure 3.30 BA 125 µM primary root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	426.91	19	22.47	1283.0	<0.001
Concentration	576.32	1	576.32	1283.0	<0.001
Varieties. Concentration	80.64	19	4.24	1283.0	<0.001

Table 21. Statistical (REML) analysis for Figure 3.31 BA 125 µM lateral root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	194.11	19	10.22	1113.5	<0.001
Concentration	289.83	1	289.83	1111.6	<0.001
Varieties. Concentration	44.09	19	2.32	1113.6	0.001

Table 22. Statistical (REML) analysis for Figure 3.32 BA 125 µM total root length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	352.66	19	18.56	1283.0	<0.001
Concentration	581.65	1	581.65	1283.0	<0.001
Varieties. Concentration	58.60	19	3.08	1283.0	<0.001

Table 23. Statistical (REML) analysis for Figure 3.33 BA 125 µM shoot length (cm).

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	1029.48	19	54.18	1301.0	<0.001
Concentration	38.47	1	38.47	1301.0	<0.001
Varieties. Concentration	32.06	19	1.69	1301.0	0.032

Table 24. Statistical (REML) analysis for Figure 3.34 BA 125 µM aerial shoot number.

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Varieties	224.79	19	11.83	795.1	<0.001
Concentration	415.40	1	415.40	797.0	<0.001
Varieties. Concentration	77.57	17	4.56	795.2	<0.001