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Research Note

The effects of introgression of the Submergence1 allele into rice cultivar IR64 on post-harvest seed dormancy and longevity

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Abstract

Introgressing the Submergence1 (*Sub1*) allele into chromosome 9 of high-yielding rice (*Oryza sativa* L.) cultivars introduced the flood-tolerant trait, but this chromosome also includes quantitative trait loci for seed storage survival. Loss in seed dormancy and viability during post-harvest storage were investigated in rice cultivars 'IR64' and 'IR64-Sub1' to test if the introgression of *Sub1* affected seed longevity. They were grown in the same controlled environment, mature seeds harvested and ability to germinate monitored during subsequent hermetic storage at 40°C with 13.5-13.9% moisture content. The overlapping patterns of loss in dormancy and loss in viability during storage were quantified well by a multiplicative model, with similar responses in the two near-isogenic cultivars: both showed almost full dormancy initially with complete, similar, loss in dormancy during the first seven days of storage, loss in viability after 43 days and 50% viability periods (p_{50}) of 26.4-28.1 days. Hence, introgression of the submergence-tolerant allele *Sub1A-1* in rice cv. 'IR64' did not affect seed dormancy or survival after harvest substantially, but duration to flowering was nine days longer and seed yield also greater in cv. 'IR64-Sub1'.

Keywords: dormancy, germination, longevity, *Oryza sativa* L., rice, seed, *Sub1*

Experimental and discussion

Seed dormancy in rice (*Oryza sativa* L.) is necessary to avoid, or reduce, pre-harvest sprouting and varies considerably amongst cultivars at maturity (Roberts, 1963; Tejakhod and Ellis, 2018). It must be lost after harvest for seeds to germinate when sown. Rice seed dormancy declines during maturation *in planta* (Tejakhod and Ellis, 2018); and

subsequently during storage *ex planta* (after ripening) at a rate dependent upon temperature (Roberts, 1965) and moisture content (Ellis *et al.*, 1983). There is no causal relationship between seed dormancy and longevity (Roberts, 1963; Miura *et al.*, 2002). Nonetheless, rice seed storage longevity (storage period for a given loss in viability) is also a function of temperature and moisture content (Ellis and Hong, 2007). In addition to the death of a fraction of the stored seeds, the surviving seeds age (Ellis and Roberts, 1981). Aged rice seeds often emerge poorly from seedbeds producing a non-uniform crop with poor growth, fewer panicles and filled grains, and lower yield (Siddique *et al.*, 1988; Yamauchi and Winn, 1996).

The widely-grown, high-quality, high-yielding mega rice cv. 'IR64' is now being replaced by newer varieties, many of which are its progeny or relatives (Mackill and Khush, 2018). Furthermore, submergence-tolerant varieties have been developed by introgressing the *Sub1A-1* allele into high-yielding parents such as 'IR64' by marker-assisted backcrossing (Septiningsih *et al.*, 2009). They can survive flooding during the vegetative phase for up to two weeks (Ram *et al.*, 2002; Das *et al.*, 2009; Sarkar *et al.*, 2009; Singh *et al.*, 2009). Submergence during the reproductive phase can reduce yield and induce pre-harvest sprouting, but less so in *Sub1*-introgressed lines than in parents (Ray *et al.*, 2017; Tejakhod and Ellis, 2018).

Marker-assisted backcrossing is an essential tool to breed new varieties with a particular phenotype rapidly (Collard and Mackill, 2008), but may result in genetic linkage between selected loci and neighbouring regions and so reduce or reinforce certain traits (Harrison *et al.*, 1987). Major quantitative trait loci (QTL) for seed viability, *qLG-9* (Miura *et al.*, 2002; Sasaki *et al.*, 2005; Shigemune *et al.*, 2008; Li *et al.*, 2012), and submergence-tolerance, *Sub1* (Xu and Mackill, 1996; Xu *et al.*, 2000, 2006), are both located on chromosome 9 in rice.

Genetic linkage between the *Sub1* locus and adjacent loci on chromosome 9 in rice has been reported after introgression (Ideta *et al.*, 1995; Neeraja *et al.*, 2007; Shao *et al.*, 2012). There is currently no evidence of potential linkage between QTLs for longevity and submergence-tolerance on chromosome 9 in 'IR64'. This is supported by the recent discovery of a large deletion in the *qLG-9* region (Kretzschmar *et al.*, 2015); and because the *Sub1* region (Xu *et al.*, 2006) does not share any candidate genes/markers with the QTL for seed viability, *qLG-9* (Sasaki *et al.*, 2015). Nonetheless, recent results from seed storage implied that introgression of the *Sub1A-1* allele might have increased longevity slightly, whether or not mother plants were submerged after flowering, and highlighted the need for further study (Tejakhod and Ellis, 2018). Rice seed survival in storage is affected by the seed production environment (Ellis *et al.*, 1993; Kameswara Rao and Jackson, 1996). Hence, any post-harvest comparison of cultivars requires the same conditions of seed production and of storage. Seeds of the two cultivars, 'IR64' and 'IR64-Sub1', were produced and then stored in the same environments to test the null hypothesis that introgression of the *Sub1A-1* allele into 'IR64' does not affect ability to germinate after storage.

Seeds of the near-isogenic Indica cultivars 'IR64' and 'IR64-Sub1' (IR07F102) from the Plant Breeding, Genetics and Biotechnology Division, International Rice Research Institute (IRRI), Los Baños, Philippines, were multiplied at the University of Reading.

The respective absence and presence of the *Sub1A-1* allele in multiplied seeds was confirmed by DNA extraction, PCR amplification, digestion with the restriction enzyme *BseNI,* gel electrophoresis and DNA sequencing of the appropriate band (Tejakhod, 2015).

Plants of the two cultivars were grown in pots in growth cabinets at 28/20°C day/night (11 hours/13 hours) with 11 hours day^{-1} photoperiod at the Plant Environment Laboratory, University of Reading, in a randomised complete block design of three blocks with eight replicates for each cultivar (48 pots in total). Plant growth procedures were as described by Tejakhod *et al.* (2018). Seeds were sown on 2 June 2014 and thinned at 70 days after sowing (DAS) to the four strongest in each pot. Phenology was recorded and a nutrient solution (Yoshida *et al.*, 1976) provided by drip-feed irrigation until two days before harvest.

Seeds were harvested by hand 44 days after anthesis, grains threshed from panicles by hand, combined from all plants within each cultivar within each block, and unfilled seeds discarded. Seed moisture content was determined by the two-stage, high-constant-temperature-oven method (ISTA, 2013) with two replicates of 2.5-3.0 g for each seed sample. Dry matter yield per pot was recorded. Thousand seed dry weight was determined from 800 seeds (of known moisture content) drawn by hand sampling.

Seeds were dried initially at 22 ± 2°C and moisture content then adjusted to 13.7 ± 0.2% by drying in a desiccator over silica gel at 20°C. Seed equilibrium relative humidity (eRH) was monitored by dew point hygrometer (Aqualab, 3TE, Decagon Devices, Inc., Pullman, USA). After equilibration for seven days at 2-4°C in an airtight glass bottle, moisture content was determined by the high-constant-temperature-oven method (ISTA, 2013).

Samples were sealed in separate laminated-aluminium-foil bags (Retort laminate, Moore & Buckle Ltd., St Helens, UK), stored in a heated incubator at 40 ± 0.5°C, and withdrawn from storage to test ability to germinate at 1-5 day intervals for up to 43 days. Three replicates of approximately 70 seeds each were sown between moist rolled paper towels [Kimberley Clark Professional, Hostess Natural Hand Towels – S Fold (Natural, 240×350 mm), Greenham Sales, UK] within loosely-folded polyethylene bags, and placed upright at 34/11°C (16 hours/8 hours) for 21 days to break dormancy and promote germination (Ellis *et al.*, 1983). Seed covering structures were removed from seeds still firm after 21 days and tests extended by seven days. Normal seedlings (ISTA, 2013) were identified, removed, and recorded.

Statistical analyses were carried out using GenStat (17th Edition, 2014, VSN International Ltd., UK). For each of seed moisture content at harvest, weight, and yield per pot, a two samples *t*-test was performed to compare varietal differences between 'IR64' and 'IR64-Sub1'.

Serial results for ability to germinate normally during storage were fitted by probit analysis in accordance with

$$v = K_i - p / \sigma \tag{1}$$

where v is probit percentage viability after p days in storage under a constant environment, σ is the standard deviation of the frequency distribution of seed deaths in time (days), K_i is the seed lot constant, and the 50% viability period (p_{50}) is the product of K_i and σ

(Ellis and Roberts, 1980). Due to considerable dormancy at harvest, a multiplicative model combining loss in dormancy and viability (Kebreab and Murdoch, 1999) was also applied:

$$g = (K_d + \beta_1 p) \times (K_i - (p / \sigma)) \qquad (2)$$

where g is probit percentage ability to germinate normally, K_d is initial probit germination (i.e. non-dormant seeds) in normal equivalent deviates (NED), β_1 is loss in dormancy (NED day^{-1}), with K_i, p and σ as above.

Cultivar 'IR64' produced flag leaves on 81 DAS, with flowering (50% anthesis) 96 and harvest 140 DAS. For 'IR64-Sub1', these development stages were each nine days later at 90, 105 and 149 DAS, respectively. The latter also flowered six days later than 'IR64' in a separate study (Tejakhod, 2015). The sensitivity of crop duration to photo-thermal environment varies amongst rice genotypes (Summerfield *et al.*, 1992). The introgression of *Sub1* into 'IR64' may have affected the expression of genes controlling phenology; this difference in the duration of the vegetative phase warrants investigation in contrasting photothermal environments.

Seed moisture content of 'IR64' was marginally greater ($P < 0.001$) at harvest than 'IR64-Sub1' (table 1). It also had slightly heavier seeds than 'IR64-Sub1' ($P < 0.01$), whereas grain yield per pot for 'IR64' was 30% less than for 'IR64-Sub1'. In an earlier investigation, however, yield per pot tended to be greater in 'IR64' (Tejakhod, 2015). This considerable difference reinforces the general view that growth chamber studies are not reliable indicators of crop yield. Nonetheless, the greater yield of 'IR64-Sub1' here was derived from a 52% increase in the number of seeds per plant which, in turn, was compatible with the longer vegetative phase.

Table 1. Moisture content, seed weight and yield of rice cultivars 'IR64' and 'IR64-Sub1' produced in a growth cabinet at 28/20°C (11 hours/13 hours).

	'IR64'		'IR64-Sub1'		P*
	Mean	(SE)	Mean	(SE)	
Moisture content at harvest (%)[†]	30.9	0.1	29.6	0.2	< 0.001
1000 seed dry weight (g) [‡, §]	21.0	0.2	19.7	0.1	0.002
Yield (g pot^{-1}) [‡, §]	102.2	9.2	146.4	3.6	0.011

* Test for significance of difference between cultivars for the independent variable shown in each row.
[†] Mean of six replicates (two per block).
[‡] Mean of three replicates (one per block).
[§] Four plants grown in an effective area of 0.0324 m^2 per pot.

Seeds showed high dormancy initially (figure 1). This was lost over the first seven days of storage with almost full germination before subsequent loss in viability over the next 36 days. The patterns of loss in dormancy and of viability were described well by the multiplicative model combining loss of seed dormancy and of seed viability (Equation 2), with positive and negative cumulative normal distributions respectively in both
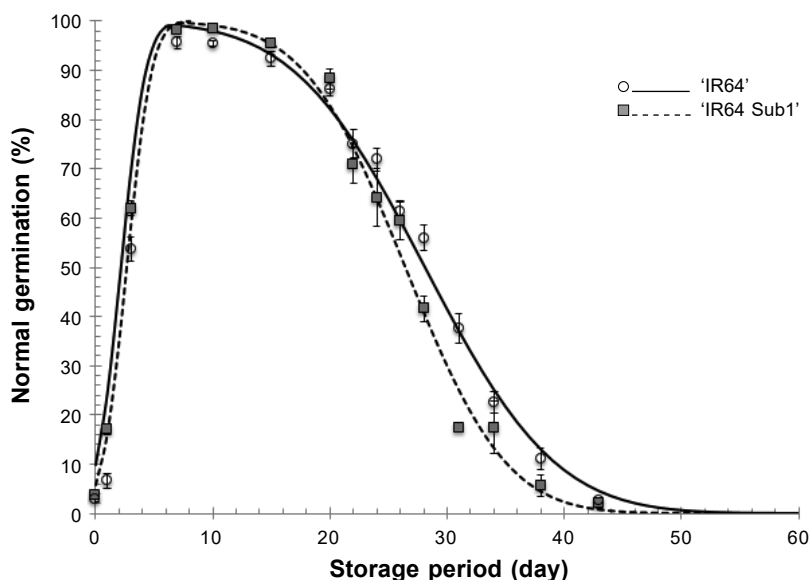
Figure 1. Changes in ability to germinate normally during hermetic storage at 40°C with 13.5-13.9% moisture content for seeds of rice cultivars 'IR64' and 'IR64-Sub1' produced in a growth cabinet at 28/20°C (11 hours/13 hours). Observations are means ± standard errors ($n = 9$, three germination test replicates each from three blocks). The fitted models describing the combined loss in dormancy and loss in viability (Equation 2) are quantified in table 2 (seed lots from all three blocks combined).

cultivars. The overall model could not be constrained to a common line for both cultivars ($P < 0.001$), nor for loss in seed viability ($P < 0.01$), but loss in dormancy (β_1) did not differ between the cultivars ($P > 0.25$) with a common value applied (figure 1, table 2). Seeds of 'IR64-Sub1' retained dormancy for marginally longer than those of 'IR64', but lost viability slightly earlier. These small differences between cultivars were of similar magnitude to those amongst blocks; comparison within blocks showed identical estimates of p_{50} in Block 1, but greater estimates in 'IR64' than 'IR64-Sub1' in Blocks 2 and 3 (table 2).

The full release from dormancy in hermetic storage over seven days at 40°C with $13.7 \pm 0.2\%$ moisture content (figure 1) is in broad agreement with the recommendation to break rice seed dormancy by sun drying to 11% moisture content and then incubating at 47°C for seven days (Roberts, 1962). The high proportions of dormant seeds at harvest in both cultivars combined with very similar patterns of loss in dormancy are compatible with both showing high resistance to pre-harvest sprouting after four days of complete submergence of plants during the reproductive phase (Tejakhod and Ellis, 2018).

That earlier study also showed good agreement of fitted values of σ with independent values provided by the improved seed viability equation (Ellis and Hong, 2007). This was not the case in the current study: σ fitted by Equation (2) ranged from 5.4 to 9.5 days at 40°C with 13.5-13.9% moisture content (table 2), much less than independent estimates of 14.4 to 22.4 days at 39-41°C with 13.5-13.9% moisture content (Ellis and Hong, 2007).

Table 2. Parameters of a multiplicative model (Equation 2) combining loss in seed dormancy with loss in seed viability of rice cultivars 'IR64' and 'IR64-Sub1', harvested from a growth cabinet at 28/20°C (11 hours/13 hours) at harvest maturity, in subsequent hermetic storage at 40°C with 13.5–13.9% moisture content and resultant estimates of seed longevity (σ and p_{50}). Estimates of β_1 (all blocks) did not differ between the cultivars and a common value is shown; those of K_d, K_i and $1/\sigma$ differed between cultivars and separate estimates are shown (see text).

Cultivar	Block (Moisture content, %)	Parameter					
		K_d (SE) (probits)	β_1 (SE) (day⁻¹)	K_i (SE) (probits)	$1/\sigma$ (SE) (day⁻¹)	σ (days)	p_{50} (days)
'IR64'	1 (13.8)	-1.87 (0.25)	0.548 (0.082)	2.88 (0.25)	-0.105 (0.009)	9.5	27.4
	2 (13.5)	-2.05 (0.26)	0.644 (0.093)	3.45 (0.26)	-0.122 (0.009)	8.2	28.3
	3 (13.8)	-1.86 (0.25)	0.697 (0.107)	3.33 (0.25)	-0.116 (0.009)	8.6	28.6
'IR64-Sub1'	1 (13.8)	-1.57 (0.22)	0.565 (0.083)	5.07 (0.45)	-0.187 (0.017)	5.4	27.4
	2 (13.9)	-1.50 (0.24)	0.583 (0.101)	3.48 (0.32)	-0.138 (0.012)	7.2	25.1
	3 (13.6)	-1.81 (0.38)	0.740 (0.169)	3.52 (0.41)	-0.130 (0.015)	7.7	27.1
'IR64'	(all)	-1.29 (0.13)	0.599 (0.042)	3.21 (0.28)	-0.114 (0.010)	8.8	28.1
'IR64-Sub1'	(all)	-1.58 (0.12)	0.599 (0.042)	3.90 (0.22)	-0.147 (0.001)	6.8	26.4

Furthermore, surprisingly (given their lower moisture content), less than the range 11.6 to 19.0 days for many more seed lots of these cultivars in the harsher storage regime of 40°C with 14.5-14.9% moisture content (Tejakhod and Ellis, 2018). Whitehouse *et al*. (2018) also reported that longevity in Indica and Japonica rices was overestimated by the independent estimates from Ellis and Hong (2007).

In conclusion, only small differences were detected in the current study in air-dry seed storage longevity (p_{50}) and release from dormancy between these near-isogenic cultivars, with p_{50} for 'IR64' > p_{50} for 'IR64-Sub1' and K_d for 'IR64' > K_d for 'IR64-Sub1' (figure 1). The latter is negligible compared with that shown between different rice cultivars produced in one environment (Roberts, 1963). Similarly, the difference in longevity detected is smaller than those amongst blocks within each cultivar (table 2). Moreover, previous research with these two cultivars provided slightly greater seed longevity for 'IR64-Sub1' than for 'IR64' in all seed production environments studied (Tejakhod and Ellis, 2018). Hence, the introgression of *Sub1* has had no detrimental effect on rice seed dormancy or longevity after harvest, but may have altered crop durations in some photothermal environments.

Acknowledgements

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