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Identifying GM crops for future cultivation in the EU through a Delphi forecasting exercise

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Abstract

This paper reports on a Delphi forecasting exercise carried out to identify crop traits that could feasibly be introduced to the advantage of arable farmers, and for the general benefit of the public, in EU member states. An expert stakeholder panel was recruited and asked for opinions on scenarios concerning the availability of GM events, and also scenarios that envisage novel crops developed using advanced technology not classified as GM. In a second round of consultation, panel members commented anonymously on opinions elicited in the first phase. Results indicate that crops with input traits most likely to become available in the EU before 2025 are HTIR maize, HT sugarbeet and HT soybean; these are already widely adopted outside Europe. The crops with output traits most likely to become available and offering benefits to consumers are winter-sown varieties of rape with reduced saturated fats, spring varieties of which are already available outside EU (notably Canadian Canola).

Keywords: forecasting, genetic modification, herbicide tolerance, insect resistance

1. Introduction

There is growing evidence that genetically modified (GM) crops have delivered substantial economic benefits for farmers, both small and large scale, as well as environmental benefits, in the limited number of countries where cultivation has been permitted (see, for example, James, 2014). With one exception, i.e. Bt maize, there is no GM crop currently permitted for cultivation in the European Union (EU) and the area under cultivation remains small, and is largely confined to Spain and Portugal with a combined total area planted of around 140,000 ha. As pointed out by the House of Commons (2015), there being slight prospect of approval for cultivation of other GM crops, there is little incentive for biotech crop developers, the great majority of whom are commercially focused, to develop crop trait combinations that are specifically targeted at agronomic conditions in the EU. However, recent political developments in the EU (e.g. the so-called Brexit decision) could signal a change in this respect.

Outside of the EU, the pipeline of new GM crops continues to grow. Stein and Rodriguez-Cerezo (2009) identified a pipeline of new GM crops of potential interest for EU arable farming awaiting field trials, and the USDA Animal and Plant Health Inspection Service (APHIS) regularly publishes a list of successful petitions for unregulated release of GM events into the environment in the USA; the 117th such petition, for a potato with blight-resistance and other properties, was approved for trials in September 2015 (APHIS, 2015).

A working group was established by the European Commission in 2007 to evaluate whether certain new techniques constitute techniques of genetic modification and, if so, whether the resulting organisms fall within the scope of the EU GMO legislation. Lusser and Davies (2013) reported on the findings of the working group, and assessed worldwide attitudes to six new plant breeding technologies (NPBTs). Crops resulting from some of the NPBTs cannot be distinguished from conventionally bred crops. Lusser and Davies (2013) considered that a global discussion of the regulation of such crops appears to be indispensable to avoid disruption of trade in the future. Hartung and Schiemann (2014) compared some of these NPBTs defined by the EU expert group with classical breeding techniques and conventional transgenic plants, and

noted that plants developed by NPBTs are often indistinguishable from classically bred plants and are not expected to possess higher risks for health and the environment.

The work reported here was carried out within the EU-funded AMIGA Project (www.amigaproject.eu) in the second half of 2015 and involved a ‘Delphi’ survey of expert GM stakeholders from various relevant sectors. In this paper, we report some results from this work which investigated the likely availability of GM arable crops in the future suited for EU agriculture and that offer benefits both to farmers and citizens in terms of agronomic, economic and environmental benefits.

The so-called ‘Delphi’ technique was largely developed by Dalkey and Helmer (1963) at the RAND corporation in the USA in the 1950s. It has become a well-used and accepted method for allowing expert opinion to help predict the future. As Hsu and Sandford (2007) say, it makes ‘sense of consensus’ in that it uses well-informed individuals to express their insights and experience on future change or developments.

The Delphi technique is an anonymous method for consensus building using questionnaires to collect opinions and data from a panel of experts (Linstone & Turoff, 1975; Martino, 1993; Young & Jamieson, 2001). The key characteristic of a Delphi process is that the data gathering has both the elements of ‘iteration’ and ‘feedback’. Subject anonymity in the process is seen as an important benefit of the process which reduces the effects of dominant individuals in a group setting (Dalkey, 1972). The first round of the investigation involves the sending out of a structured open-ended questionnaire to a panel of experts. In the next round (or iteration), the panel is sent a second questionnaire which contains both their original answers (or feedback) and a summary of those of the other panel members. Further iterations are sometimes carried out.

While the Delphi technique has been widely used in many technological, social and political fields, it has not been much used in the area of food and agriculture. Nevertheless, Fearne (1986) used the Delphi technique to forecast agricultural policy decisions in the EU, Menrad et al. (1999) used it to examine future trends in biotechnology in the EU, as did Cunha and Swinbank (2009) when exploring what had prompted the

European Commission to reform the Common Agricultural Policy in 1992, 1999 and 2003. Also, Ilbery et al (2004) used it to study food supply chain developments in rural lagging regions and Kenyon et al. (2008) used it for scoping the role of agriculture in flood management. More recently, Stebler et al. (2015) used a Delphi panel to identify, and weight, the prioritisation of the management of zoonotic diseases in Switzerland and the technique was used to evaluate vegetation management strategies under electric power lines by Dupras et al. (2016).

2. Method

During the ten years from 2015, what crop-trait combinations from elsewhere will be made commercially available in the EU, or developed specifically for Europe, that could offer potential benefits to EU agriculture and/or society at large, should the policy environment become permissive enough to allow their cultivation was asked. For the work reported here, crop traits were identified as providing potential benefits in terms of:

- herbicide-tolerance (HT)
- insect-resistant (IR)
- frost-tolerance
- combating the effects of pests and pathogens
- improving bread-making properties of wheat
- enhancing nutritional properties of oilseeds
- other traits, such as bruise-resistance, of value in the food chain.

This shortlist of crop-trait combinations was selected by a team from the afore-mentioned EU FP7 AMIGA project, from various databases and journal sources, including Riccroch and Henard-Damave (2016), Hefferon (2015), De Steur et al. (2015) and APHIS (2015).

To carry out the Delphi study, a panel of experts had to be recruited representing stakeholders with various professional roles in crop research and development, arable farming, crop protection and farm management. The recruitment was made from a database of participants in recent UK and European technical meetings and research activities, and included representation of experts from outside Europe.

An explanatory letter and a one page sheet for recording consultees' estimates and opinions on availability of GM crops for areas in the EU were sent out to 212 people electronically as the first round of the consultation exercise. This initial list was drawn up in four ways: from lists of attendees at conferences on GM crops; authors of appropriate papers in the journal AgBioForum; authors of papers referred to in relevant papers in AgBioForum; and people the current authors knew to have knowledge and interest in GM crops. Some 43% of the initial list were university academics, 20% were from independent research institutes, 20% were government officials and 17% from a commercial company background. Approximately 24% were located in North America, 68% in Europe and the remaining 8% in the rest of the world.

To increase response rate, a reminder e-mail was sent after 30 days and a total of 51 replies were received: 26 were sufficiently complete for the respondents to be retained as the panel. Of these: 10 were academics; 9 were from a commercial background; 4 were from a research institute; and 3 were what can be described as Government officials.

The expert panel were asked for their opinion on the following GM crop-trait combinations, divided into two groups:

- Ten GM crops with input traits conferring advantages to the farmer: **winter oilseed rape (OSR)** – HT; **potato - IR**; **potato - pathogen tolerant**; **wheat - drought tolerant**; **wheat - frost tolerant**; **barley - frost tolerant**; **sugarbeet - HT**; **soybean - HT**; **maize - drought tolerant**; **maize – HT** and IR.
- Seven GM crops with output traits modifying the characteristics of the harvested product: **wheat** - with improved bread making properties; **wheat** - with higher dietary fibre; **wheat** - with reduced levels of protein linked to celiac disease; **soybean** - with improved nutritional profile; **OSR** - producing Omega 3 oils as a dietary supplement; **OSR** - with a lower saturated fat content; **potato** - with resistance to bruising.

Respondents were asked for their opinion as to whether these crops would be available before 2025. A 6 point Likert scale was used, from 0 being 'not likely' to 5 'very likely'. They were then asked their opinion

on the likely effect of the adoption of the crops on farmers' costs, yields and prices in percentage terms as compared with equivalent conventional 2015 crops. The second round consultation was sent to panel members 60 days after the original mailing and members were shown their original estimate and also the mean of the panel's estimates. They were then invited to confirm or amend their original opinion or estimate; of the 26 panel members, 7 made revisions to their original estimates.

3. Results

3.1 Introduction

The estimates of the panel of experts on likely availability of the 'new' crops with input traits being available before 2025, following two rounds of consultation, are shown in Table 1, and for output traits in Table 2.

Opinions on the likely effects of the crops on farmers' costs, yields and prices compared with conventional 2015 crops are also shown. They represent the mean scores for the whole panel for both rounds of consultation, together with a measure of the change in the variability found in these estimates from first round to second round, expressed as the change in standard deviation (SD) score. As can be seen from Table 1 and Table 2, when the panel's round one and round two estimates were tested for differences, no statistically significant changes were found.

When SD change scores are negative, this implies that the SD of the sample estimates (i.e. the variation between individual estimates) is decreasing between the rounds as the panel closes in on consensus. When SD change estimates are small, this means that there is relatively little change in the SD estimates between rounds and this, in turn, implies that convergence has already largely been reached and that further iterations would only yield very small marginal improvements in convergence. As can be seen from Tables 1 and 2, the SD change scores are generally negative and small, implying that there would be only limited benefit, in terms of convergence of opinion, from additional iteration rounds, even if the panel would be prepared to take part in the study again.

3.2 GM crops with input traits

Looking first at the availability estimates in Table 1, the main point to note is that all estimates are relatively low. Based on the ranking scale used, a score of 5 represents 'very likely', while a rank of zero represents 'not likely'. On this basis, a mid-point rank of 2.5 might be interpreted as a 50% likelihood estimate, i.e. the zone of uncertainty. Few of the likelihood estimates rise above this mid-point, suggesting an expectation of relatively low likelihood of any of the crop-trait combinations being available for use by EU farmers by 2025. The panel felt that the crop-trait combination most likely to be available to EU farmers by 2025 is maize with stacked traits for herbicide tolerance and insect resistance, with a mean rank score of 2.76. The crops thought next most likely to become available are HT soybean with a rank score of 2.48, then HT sugar beet (2.39) and pathogen tolerant (PT) potato (2.27).

Table 1 also reveals that the trait that the panel thought least likely to become available before 2025 was frost tolerance, both for barley (0.74) and wheat (0.78). Also given very low likelihood rankings are drought tolerance in wheat (1.3) and insect resistance in potato (1.33).

Input-side traits are expected to offer financial benefits to the farmer from either reduced input costs, especially crop protection costs, and/or increased revenues, through improved (or protected) yields. Table 1 shows that the panel anticipated cost savings in six out of ten of the crop-trait combinations, but increases in production costs in the remainder. Costs savings ranged from 4.47% to 5.89%, a relatively narrow range, these being somewhat larger in magnitude than the expected cost increases, which range from 0.55% to 2.38%.

The crop-trait combinations offering the largest savings in input costs are PT potato (5.89%), HT winter oilseed rape (5.74%) and HT soybean (4.93%). At the other end of the spectrum, the panel thought that drought tolerant wheat would raise farmers' costs by 2.38% and frost tolerant barley by 1.05%.

It is notable that the crop traits expected to increase costs are frost and drought tolerance. This does make perfect sense because, with the possible exception of irrigation, these traits do not replace any inputs, such as chemical sprays, but they may incur higher seed costs. However, these traits may still prove to be financially

advantageous if their yield protection benefits, in years when weather conditions are unfavourable, offset the additional seed costs when averaged over the longer term.

As Table 1 shows, the highest and lowest anticipated yield improvements are both recorded for potatoes, with yield improvement estimated to be just 3.75% for IR potato, and as much as 9.14% for PT potato. This result suggests a panel consensus that current yield losses resulting from insect pests, such as Colorado and Flea Beetles, are considerably lower than yield losses from diseases such as Brown Rot and Late Blight. Drought tolerance is estimated to offer greater potential yield benefits than the average, at 8% for wheat and 6.73% for maize, while frost tolerance traits are estimated to offer slightly below average yield improvements at 4.97% for both wheat and barley.

3.3 GM crops with output traits

In terms of crops with output traits, Table 2 shows that all likelihood of availability ranks are again low, signalling a generally low expectation by the panel that any of the traits will be available to EU farmers by 2025.

The crop offering enhanced nutritional properties thought most likely to be available is oilseed rape, i.e. rape producing Omega 3 oils as a dietary supplement, with a mean rank score of 2.13, followed by rape with a lower saturated fat content (2.08). Soybean with improved nutritional profile was ranked some way behind these (1.75).

The crop offering altered nutritional properties viewed by the panel as least likely to be available is wheat, in particular wheat with reduced levels of protein linked to celiac disease (1.04), wheat with higher dietary fibre (1.08), followed by wheat with improved bread-making properties (1.26).

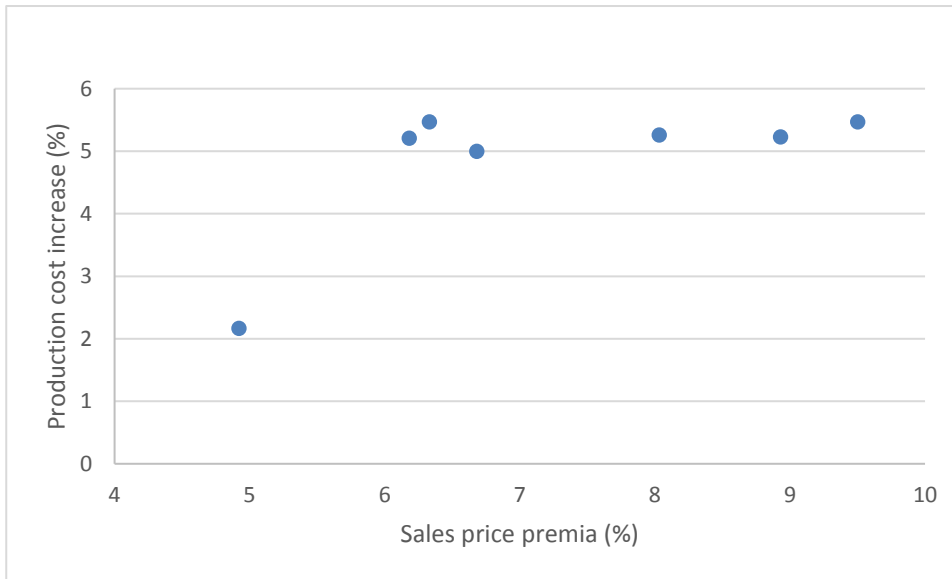
The panel anticipated that the cultivation of all of the crop-trait combinations under consideration will incur increased costs compared to the conventional equivalent (see Table 2). These cost increases will be due, almost in their entirety, to higher seed costs, as biotech companies attempt to recoup seed development costs.

Interestingly, the crop viewed as being the least likely to be available in GM form, i.e. wheat with improved bread-making properties, is also expected to incur the largest increases in production (seed) costs, i.e. 5.47%. At the other end of the scale, the output crop-trait the panel anticipated having the lowest cost change for farmers, was potato with bruising resistance (2.17%).

The nutritional profile changes identified for GM crops in this study were viewed by the panel as desirable and so all were expected to offer a price premium to the farmer. The crop-trait combination expected to offer the highest price premium, compared to its conventional counterpart, is wheat with reduced levels of protein linked to celiac disease, with a potential price premium of 9.5%. Oilseed rape producing Omega 3 oils as a dietary supplement was also expected to offer a substantial premium (8.93%). The crop with the lowest estimated premium was potato with resistance to bruising (4.92%). This relatively low premium may be due to the fact that this new trait offers no direct benefit to consumers, but rather benefits to intermediaries through reduced losses during transport and storage and, perhaps also, farmers during harvest.

Economic logic suggests that the price premium attaching to seed costs will be related to the size of the expected price premium available on the harvested crop itself. The larger the sales price premium, the larger the premium that farmers will be willing to accept on the price of seed. Figure 1 shows a test of the extent to which the panel of experts has recognised this principle, whether consciously or not, in providing their estimates.

Figure 1. Relationship between the panel of experts' predicted sales price premia and input cost increase for GM crops with output traits.



As Figure 1 shows, while there seems to be some reflection of the likely lower sales price of the bruise-resistant potato in the estimated increase in production costs, there would seem to be no recognition of this relationship in the input cost estimates of the other crop-trait combinations. The basis on which these production cost increases have been estimated is, therefore, uncertain, but may reflect the panel’s associated average experience for GM crops in other geographical regions.

3.3 Other possible crops and crop-trait combinations

When given the opportunity to suggest other crop-trait combinations that might be both available to EU farmers and offering wider societal benefits, there were only relatively few suggestions by panel members and these were dominated by crops with various types of biofortification. The rationale for such suggestions must be influenced, in part, by GM events in the development pipeline at the time of study but also, perhaps, by an assumption that there might be a more positive reception for such crops by EU consumers due to their health-promoting qualities. However, the generally negative expectations about future GM policy on authorisations in the EU was also apparent in these responses. Accordingly, some panel respondents declined to suggest any new or novel GM crops, but rather pointed to the products of NPBTs which do not use transgenesis, such as CRISPR, as being more likely to be available to EU farmers in the near future.

4. Discussion and conclusions

The study reported here did not attempt to rank GM arable crops in terms of potential economic or public

health benefits. It aimed, simply, to reveal stakeholders' expectations about a number of specific crop traits, and to do even that without attempting to assess any benefits arising from combining or 'stacking' of traits in any one crop, with the exception of herbicide-tolerant insect-resistant (HTIR) maize.

The headline outcome of this reported Delphi survey of GM stakeholders is the somewhat low expectation that any of the 17 GM crop-trait combinations under consideration will be available to farmers in the EU by 2025, with the maximum likelihood of availability placed at around 50%. Because a broad range of crops and GM traits was under consideration, it can be inferred that there was only a modest expectation amongst the stakeholders consulted that any GM crops will be available to EU farmers within the time-frame considered. (However, at the time of consultation (2015) the so-called Brexit decision had not yet happened.) There are three possible reasons for these low 'availability' estimates. First, crop-trait combinations may still be at early stages of development and so may not be available for marketing within the time-frame considered. Second, the policy environment is expected to remain challenging for GM releases in the EU (effectively maintaining the current moratorium) even by 2025 and, third, there is such uncertainty surrounding the issue of availability that the stakeholders consulted were not able to arrive at a consensus.

Of the input crop traits on which the panel gave opinions on likely availability, the highest 'scoring' crops were HTIR maize, HT soybean and HT sugarbeet; all these crops are already widely adopted outside Europe (James, 2014). Other relatively strong scoring crops were HT winter OSR and pathogen tolerant (blight resistant) potato. Nevertheless, HT winter OSR is not available at the time of writing, and pathogen-tolerant potato was not available for any commercial cultivation until it was approved for unregulated release in the USA in September 2015 (APHIS, 2015). Most of the input side crop traits were expected to offer financial benefits to farmers from either reduced input costs (e.g. crop protection costs) and increased revenues through improved or protected yields.

Of the output crop traits, the highest scores for likely availability were for winter OSR with dietary Omega 3 oils and with low saturated fat content; Canola (a spring variety of OSR) with high monounsaturated fatty acid (MUFA) has long been available (Kris-Etherton et al., 1999) and the health benefits are well-attested

(Astrup et al., 2011). Whilst the panel considered all these crop-trait combinations would incur increased costs to farmers (largely through higher seed costs compared to the conventional equivalent), all were thought to offer a price premium to farmers.

Uncertainty related to regulatory acceptance of all genetically altered crops overshadows the prospect for introduction of output crop traits with real benefits for both consumers and farmers. Strenuous efforts are being made within EU to achieve consensus on the regulatory environment. A press release from the European Commission (2015) summarised the situation: ‘Two different consultancies evaluated the existing GMO legislation between 2009 and 2011 focusing on GMO cultivation and GM food and feed aspects. ... Stakeholders and competent authorities expressed support for the main objectives of the legislation, such as protecting health and the environment as well as creating an internal market which remains consistent with the needs of society’. The evaluation reports also confirmed that many recent actions of the Commission were on the right track.

However, some adjustments were deemed necessary by the Delphi panel to better implement the existing legislation, for example:

- the authorisation system could be more efficient;
- GMO cultivation would benefit from more flexibility; and
- risk assessment process would benefit from further harmonisation.

Following evaluation recommendations, the Commission has launched the following actions:

- a proposal for increased flexibility on GMO cultivation;
- technical information on the socio-economic implications of GMO cultivation;
- reviewing and transforming the risk assessment guidelines for food and feed and environmental release into legal documents approved by EU countries;
- reinforcing of environmental monitoring;
- harmonised sampling and testing for low level adventitious presence in food;
- assessing of new plant breeding techniques; and
- stepping up communication on GMO issues.

The European Commission (2015) press release omits direct mention of management of coexistence of GM and conventional crops, and the topic of coexistence was not specifically included in our Delphi study. However, experts agree that decisions within EU as to coexistence issues will have a major impact on availability in Europe of novel traits introduced elsewhere (e.g. Beckmann et al., 2006; Messéan et al., 2006; Demont & Devos, 2008; Devos et al., 2009; Messéan et al., 2009).

In the longer term, all forms of crop development are benefitting from genome sequencing and the associated acceleration of introduction of beneficial crop traits; for example, a consortium collaborated to sequence the potato genome (Potato Genome Sequencing Consortium 2011), and a number of genome editing tools have been developed (Urnov et al., 2010; Belhaj et al., 2013; Joung & Sander, 2013). Although relatively new, techniques such as CRISPR are already being hailed (for example, see Belhaj *et al*, 2013 and Ledford, 2015) as the future industry standard tool for biotechnology, thereby supplanting the position of GM in plant breeding. While these NPBTs are currently still being debated by advisory bodies and regulatory authorities in the EU (Tagliabue, 2016), there is the possibility that, because they produce plant gene modifications that are indistinguishable from both conventional breeding and chemical and physical mutagenesis approaches, they will be excluded from the scope of GM legislation such as Directive 2001/18/EU on Deliberate Release of Genetically Modified Organisms. This would make releases of such crops to the EU market much more routine than at present. Whilst a formal European Commission decision on which NPBTs, if any, are to be defined as GM was likely before the end of 2015, according to a Special Report from EurActiv (2015), no such decision has yet been taken.

The study discussed here has confirmed, and to some extent quantified, the likelihood of forthcoming benefits for consumers, farmers and the wider rural economy from a selection of crop traits developed as a result of investment in crop breeding, and based on more detailed understanding of crop genetics. If the EU can agree an arrangement to permit cultivation of some, at least, of the crops developed using new plant breeding technologies, economic, environmental and other benefits will become available to EU citizens and, especially, to arable farmers. Such benefits could help ameliorate the pressure on the rural economy in the EU from the next reform of the Common Agricultural Policy which will, almost certainly, result in less

financial support to this sector.

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Table 1. Experts' views on various GM crops with input traits being available before 2025 and, if available, the likely effect of adopting the crop on farmers' costs and yields obtained.

	Mean availability score ¹			Mean farmers' cost change (%)			Mean farmers' yield change (%)		
	First round	Second round ³	SD change ²	First round	Second round ⁴	SD change ²	First round	Second round ⁴	SD change ²
Winter oilseed rape - herbicide tolerant	2.17	2.17	-0.01	-6.10	-5.74	-1.83	4.60	4.43	-0.49
Potato - insect resistant	1.38	1.33	0	-4.55	-4.47	-3.74	3.85	3.75	-1.34
Potato - pathogen tolerant	2.23	2.27	-0.02	-6.38	-5.89	-2.95	9.26	9.14	-0.98
Wheat - drought tolerant	1.39	1.30	-0.20	2.55	2.38	-0.48	6.85	8.00	-1.08
Wheat - frost tolerant	0.91	0.78	-0.14	0.16	0.55	-0.83	3.97	4.97	-0.98
Barley - frost tolerant	0.87	0.74	-0.15	0.68	1.05	-0.84	3.97	4.97	-0.98
Soybean - herbicide tolerant	2.40	2.48	-0.07	-5.75	-4.93	-2.33	4.28	4.07	-1.30
Sugarbeet - herbicide tolerant	2.39	2.39	0	-5.66	-4.70	-2.52	4.45	4.19	-1.15
Maize - drought tolerant	2.13	2.04	-0.21	0.68	0.80	-1.33	6.08	6.73	-1.17
Maize - herbicide tolerant and insect resistant	2.72	2.76	-0.03	-5.25	-4.90	-1.38	6.81	6.45	-1.30

Notes:

¹ where 0 = 'not likely' and 5 = 'very likely'.

² SD change is SD value in second round minus value in first round.

³ when differences in first and second round scores were tested for statistical significance using Wilcoxon's matched pair signed ranks test, no significant differences were found.

⁴ when differences in first and second round cost and yield changes were tested for statistical significance using the Students' t test, no significant differences were found.

Table 2. Experts' views on various GM crops with output traits being available before 2025 and, if available, the likely effect of adopting the crop on farmers' costs and prices for the crops received.

	Mean availability score ¹			Mean farmers' cost change (%)			Mean farmers' price change obtained (%)		
	First round	Second round ³	SD change ²	First round	Second round ⁴	SD change ²	First round	Second round ⁴	SD change ²
Wheat - with improved bread-making properties	1.17	1.26	-0.05	5.29	5.47	-0.20	6.26	6.33	-0.03
Wheat - with higher dietary fibre	1.13	1.08	-0.43	5.03	5.21	-0.19	5.56	6.18	0.50
Wheat - with reduced levels of protein linked to celiac disease	1.13	1.04	-0.04	5.29	5.47	-0.18	9.06	9.50	-0.10
Soybean - with improved nutritional profile	1.75	1.75	0	5.13	5.26	-0.18	7.47	8.03	0.07
Oilseed rape - producing Omega 3 oils as a dietary supplement	2.08	2.13	-0.02	5.39	5.23	-0.16	9.21	8.93	-0.75
Oilseed rape - with a lower saturated fat content	2.08	2.08	-0.03	4.87	5.00	-0.19	6.63	6.68	-0.07
Potato - with resistance to bruising	1.70	1.65	-0.02	2.36	2.17	-0.33	5.17	4.92	-1.33

Notes:

¹ where 0 = 'not likely' and 5 = 'very likely'.

² SD change is SD value in second round minus value in first round.

³ when differences in first and second round scores were tested for statistical significance using Wilcoxon's matched pair signed ranks test, no significant differences were found.

⁴ when differences in first and second round cost and yield changes were tested for statistical significance using the Students' t test, no significant differences were found.