

## Letters

# Overexpression of *epsps* transgene in weedy rice: insufficient evidence to support speculations about biosafety

The recent paper published in *New Phytologist*, Wang *et al.* (2014; this issue pp. 679–683), purports in its title that ‘A novel 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase transgene for glyphosate resistance stimulates growth and fecundity in weedy rice (*Oryza sativa*) without herbicide’ and in the paper claims that weedy rice expressing the transgene is more competitively fit than the wild type in the absence of glyphosate treatment. While this may be so, there is a lack of evidence in the paper that the transgene confers glyphosate resistance, or that the transgenic weedy rice is more competitively fit than its wild type. As this paper has generated extensive media coverage facilitated by the authors, we feel it imperative to analyze the paper in depth to ascertain whether the claims made in title, summary, and text have been met, whether criteria necessary for publication have been achieved, as well as whether the media reportage was justified in the form released by the authors.

In many respects, the findings are not as novel as the authors imply. This is not the first instance when genes from a crop have increased the rate of growth and/or fecundity of a related weed with which the crop can cross-pollinate. Many such cases have been discussed before (Ellstrand, 2003). Whenever yield-enhancing traits are introduced into crops they can quickly introgress in related infesting weeds. For example, weedy *Aegilops* spp. growing in or near wheat fields typically introgress wheat genetic material (Weissmann *et al.*, 2005), have larger flag leaves, ears, and grain than the same *Aegilops* spp. in wild habitats (Rehman *et al.*, 2006; Arrigo *et al.*, 2011). Indeed after disease resistance was crossed into a US wheat from a nonindigenous *Aegilops* spp., it quickly introgressed from wheat into indigenous *Aegilops cylindrica*, a major weed in parts of the United States (Perez-Jones *et al.*, 2006). Most crop-related weeds, and especially weedy rice, have co-domesticated with the respective crops. There is considerable evidence that most weedy rice strains derive from de-domestication of cultivated rice and are not derived directly from the wild progenitors (Gross *et al.*, 2010; Lawton-Rauh & Burgos, 2010; Thurber *et al.*, 2013, and references cited therein). Thus, weedy rice fecundity has increased through the ages because cultivated rice yield has increased. None of this very relevant information was intimated or cited, and the authors made it appear as if it is novel that a trait introduced in a crop enhanced and raised fecundity when it introgressed to a related weed.

A basic tenet of published science is that other scientists should be able to reproduce the experimental findings, based on the Materials and Methods section of peer-reviewed papers. This was not the case here. Particularly crucial, no sequence data were provided or deposited at EMBL/GenBank for the transgene construct used. The authors say they used a modified rice *epsps*, but there was no information provided on the sequence modifications, or its protein products  $K_m$  or  $K_i$  for glyphosate, yet they claim that the modified gene confers resistance to glyphosate. No data are provided that demonstrate resistance to glyphosate or its extent, nor is a reference given to any peer-reviewed study where such data are presented, despite implying that such a critical and influential connection is supported by this study. They do show a doubled level of the EPSPS protein. In other cases where there was a doubled level of EPSPS expression (Boerboom *et al.*, 1990; Baerson *et al.*, 2002) there was only a *c.* seven-fold increase in the  $I_{50}$  resistance level to glyphosate, which is usually not enough for commercial use in the field. They say that the gene was described in a published paper (Xu *et al.*, 2002) (correct citation given here and not in the manuscript in question). This paper (Xu *et al.*, 2002) only presents data on cloning the rice *epsps* gene and presents nothing about its modification. Is this the gene the authors used to produce the transgenic rice in question, which was then further crossed to weedy rice? They cite no paper on the original transgenic cultivated rice containing this gene nor on the variety they used. Additionally, there is a surprising paucity of molecular data about the transgenic rice plants, for example, there is an absence of Southern blot data, resequencing hybrids to confirm identity, gene insert number, number of transgenic events recovered, the variation of gene expression and phenotypes among the transgenic events, or even enough gene expression analysis of the glasshouse-grown plants to be certain of increased transgene expression. However, we can assume here that transgenic plants had higher expression. In some places in the paper, the authors state that the overexpressed *epsps* gene in the transgenics is the ‘native rice *epsps* gene’ (e.g. the Summary), and in other places they confusingly refer to it as a modified gene. In the Materials and Methods section, they state that the transgene is different from the native gene in size (700 and 1000 bp), but there is no sequence information on how it is different, or even if it includes the entire coding sequence. Since the entirety of the findings of Wang *et al.* rests on the material composition of the transgenic plants, we do not understand how the findings could be validated or replicated by others in rice or another species of plant and thus how this paper can be seen as a valid contribution deserving publication.

No data are given on the fecundity of the transgenic rice vs the parent variety. Did all cultivated rice transformants give such an increase or just one – that is, is this a positional effect that has nothing to do with the gene inserted? There is no sequencing and mapping of flanking regions that would supply insights into this

possibility. The absence of molecular data on the transgenics and the absence of data on the magnitude of yield increase conferred to the cultivated rice by the transgenic trait are needed as a baseline to compare with the yield increment it confers on weedy rice. This would place the data on the increase in fecundity in weedy rice in the perspective of whether this is just analogous to other cases where yield increases in crops become introgressed into their related weeds. The paper would be quite novel if the transgene insertion resulted in a significantly higher increase in fecundity in the weedy rice than the cultivated wild type, but this is not supported by the data presented.

The authors competitive fitness experiment – a key to the paper – is flawed. They used transplants spaced at  $25\text{ m}^{-2}$  to measure competition between the transgenic and nontransgenic strains, without a phase of self-thinning, one of the most critical stages of intra-specific competition. It is the balance between inter-/intra-specific competition that defines the relative competitive ability of a species growing in mixture with another (Boddy *et al.*, 2012). Thus, their experimental procedure is highly artificial and probably not relevant to field conditions considering that under typical field conditions the density of weedy rice at the early seedling stage could be  $c. 1000\text{ plants m}^{-2}$ . After self-thinning, a heavily weed-infested field reaches maturity with  $c. 50\text{ plants m}^{-2}$  of cultivated rice and  $250\text{ plants m}^{-2}$  of weedy rice, that is, 75% of the weedy rice succumbed to intra-specific self-thinning competition at very close spacing.

Relative competitiveness is just one component of the overall ecological fitness of individuals. Wang *et al.* only measured fitness of the transgenic hybrid weedy rice in competition with the nontransgenic hybrid at  $20\text{ cm}$  spacing that is,  $25\text{ plants m}^{-2}$ , which is not close enough to show a competitive fitness difference. This is borne out in published studies, for example, Boddy *et al.* (2012), and many studies cited therein (but not cited by Wang *et al.*, 2014). Wang *et al.* used an 11 : 1 ratio, which is unlikely to occur in the field because much of the weedy rice in a field experiment should be wild type, due to the very low outcrossing of rice as well as emerging dormant weedy rice from previous seasons. Studies involving different densities and ratios (replacement series) of wild type and transgenic, or having shorter distances between plants, which enhances competition that are typical of such ecological experiments (Boddy *et al.*, 2012) were not performed. The redundant analyses of variance and the figures referred to actually say nothing about enhanced fitness of the hybrids. Thus, despite the claim in the title, it is doubtful that there are any significant data in the paper on the enhanced competitive fitness of the transgenic weedy rice. We can only conclude that the transgenic material has enhanced fecundity.

The data on tryptophan overproduction seem solid – but the missing data would help in understanding the significance of their results. There are many ways to obtain tryptophan overproduction nontransgenically – for example, selection for mutants resistant to methyl tryptophan. Would the authors then say that the movement of such nontransgenic mutations would ‘have broad implications for biotech risk assessment’? Is only the movement of transgenes a worry, as the authors strongly imply, or should one worry about all novel traits as the Canadian regulatory authorities do with their

product-oriented (not process-oriented) regulation (Ammann, 2014)?

The paper does mention that there are ways to ameliorate transgene flow, should it pose a risk – and cites papers to that effect. They repeatedly emphasize the gene flow problem throughout the paper but the ‘minor’ solution does not appear in the Summary which is meant to abstract the whole paper, including the concluding take-home message. The possibility that such transgene flow could be controlled was not mentioned in any of the authors’ press releases (e.g. <http://researchnews.osu.edu/archive/geneflow.htm>) nor in their interviews (Qiu, 2013). This is probably because such transgene flow control was sufficiently well hidden such that the journalists who may have read the actual paper might not discover this or overlook the significance. Thus, besides the title and the summary not supplying data on many of the contentions, the summary and the conclusions are at odds with each other.

There is no statement that the experimental material used to support the paper’s conclusions is available to any scientists wishing to further validate the conclusions based on the data presented, as required by many journals. Of even greater importance, as we discuss earlier, the paucity of molecular data renders the experiments impossible to reproduce. This lack of critical information means that there is little opportunity for independent validation of the presented results and no possibility for follow-up directly addressing the significant issues discussed regarding transgene movement in the future. This is especially important given that the media immediately interpreted and summarized the results making strong assertions. Clearly this was either based only on the misleading title and overstated summary, and/or on a press release by an author (<http://researchnews.osu.edu/archive/geneflow.htm>). There was non-critical acceptance of their conclusions in the paper, even in *Nature News* (Qiu, 2013) and reproduced verbatim in *Scientific American*. The journalist assumed that the rice and weedy rice were herbicide resistant and the weeds with the transgene were more reproductively fit. The press release from a co-author (Qiu, 2013) echoes these contentions. Such reportage with the resulting hype, dealing with agricultural biotechnology, often influence public perception and policy far beyond the scope of the published study and thus directly impact on the potential benefits of specific transgenic crops. In this case, there is not only insufficient evidence provided in the study to justify the conclusions, but there is insufficient description of the materials used in the study to allow the scientific community to try to replicate, interpret, or even understand, the study.

In summary, to directly quote a recent *Nature* Editorial on the potential misuse of inappropriately presented and described work on genetic studies of human intelligence and behaviour: ‘Scientists have a responsibility to do what they can to prevent abuses of their work’. To do so: scientists must ‘First: be patient. Do not speculate about the possibility of finding certain results, or about the implications of those results.’ ‘Second: be accurate. Researchers should design studies on the basis of sound scientific reasoning.’ ‘Third: be sensitive. Even if scientists have truly honourable intentions, they must realize how easy it can be for studies ... to seem self-serving’ (Anonymous, 2013).’ We do not believe that any of these simple criteria have been met in the present instance.

Indeed, the authors' interviews and press releases appear to promulgate their speculative over-interpretation of very limited data.

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**Key words:** critique, EPSP synthase transgene, glyphosate resistance, *Oryza sativa*, Wang *et al.* (2014).

Editorial Note: the Letter from Gressel *et al.* states that sequence data were not supplied in Wang *et al.* (2014), despite this being a requirement of the Journal. In light of this, *New Phytologist* has taken steps to ensure that authors must, if their paper includes new sequence data, provide the relevant accession numbers. In addition, we have made our policy on data deposition explicit by amending our Author Guidelines. These now state that *New Phytologist* requires authors to make their data available to readers and interested parties upon reasonable request.