

Evaluation and Licensing Opportunities

For further information on this technology and evaluation / licensing opportunities please contact:

Dr Jan Chojecki
ajsc@pbltechnology.com
Tel: +44 (0)1603 456500

Tech ID: 13.561

Patent Literature

Grants: AU 2014308078,
ZA 2016/00915

Publications: WO2015/022192,
EP 3033354, CN 105612171 A,
US-2016-0194651-A1

DA2 Gene

Increased seed size, plant growth and grain yield when downregulated together with da1 mutants

The Da1 gene (PBL Tech Id 07.436) is known to be involved in control of the size of seeds and other plant organs, and ultimately yield, though effects on proliferation and growth of cells. Mike Bevan and Yunhai Li showed that dominant negative loss-of-function mutations of Da1 caused a marked increase in seed and organ size, and that this effect was synergistic with mutations that abolish the function of the E3 ubiquitin ligase EOD1/BB. Now, Li (at IGDB, Beijing) and Bevan (JIC) have together found another gene, DA2, which also acts synergistically to control seed/organ size, in a mechanism that is independent of EOD1/BB.

DA2, like EOD1/BB, encodes a RING-type protein with E3 ligase activity, and in WT Arabidopsis plants it limits seed size by restricting cell proliferation in the maternal integuments of developing seeds. Overexpression of DA2 in transgenic plants causes decreased seed size. However, mutants of DA2 have increased seed size. DA2 has an orthologue in rice, GW2, that is already known to be involved in control of grain width and grain weight (Song et al, 2007, Nature Genetics 39 (5) 623-630), and as with Da2 its loss of function increases cell proliferation and eventual seed size. However, these latest findings of Li and Bevan demonstrate the **synergistic effect of combining DA2 mutants or downregulation of DA2 with da1** dominant negative loss-of-function genotypes to get a much enhanced positive effect on growth, compared to the effects of either genotype alone. Further, combining with loss of EOD1/BB function increases the positive effects even further, indicating that DA2 and EOD1/BB act on DA1 independently of one another.

The inventors have shown that overexpressing the rice GW2 gene in Arabidopsis causes decreased seed size, indicating that the technology will be applicable readily in different species. They have identified DA2 orthologues with significant homology, outside of the conserved RING domain, in crops such as maize, rice, barley, soybeans and oilseed rape.

Loss of DA2 function has been studied in a T-DNA mutant line of Arabidopsis, da2-1, which shows increased seed size, without compensating reductions in seed number. Da2-1 mutants also were taller than WT at maturity and had larger flowers and leaves and overall increased biomass, each of which is due to increased cell numbers rather than increased cell size. Reciprocal crossing experiments indicate that DA2 acts maternally to affect seed size. Expression studies show that DA2 has a similar expression pattern to DA1, being expressed wherever cell proliferation is occurring during plant development. Furthermore, the inventors have demonstrated not only that DA2 interacts with DA1 both in vitro and in vivo, but also they have identified physically how it interacts, which informs approaches for modifying DA2 to prevent its interaction with DA1, as an alternative to down-regulation strategies.

Applications:

- Increased seed size, and grain yield
- Increased cell proliferation – increased vegetative or storage organ size/capacity
- Increased plant biomass
- GM and non-GM approaches

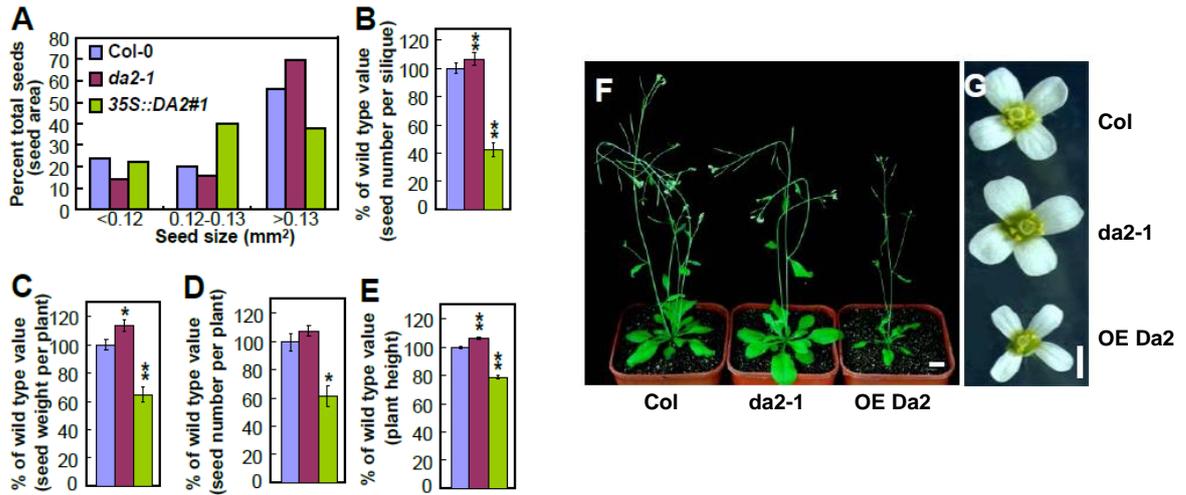
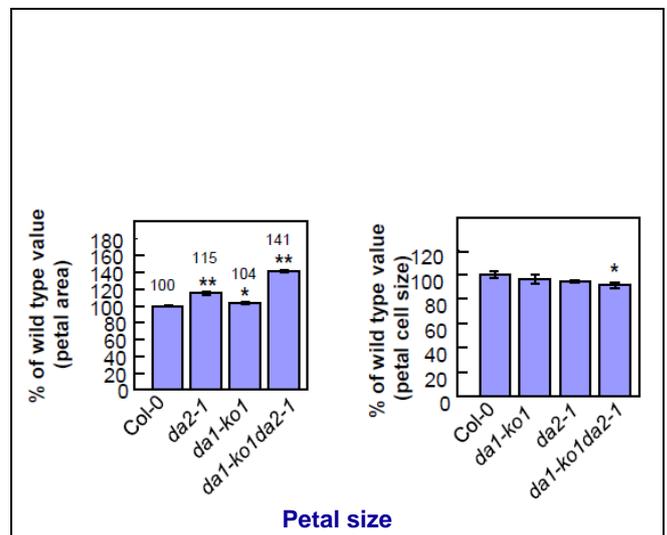
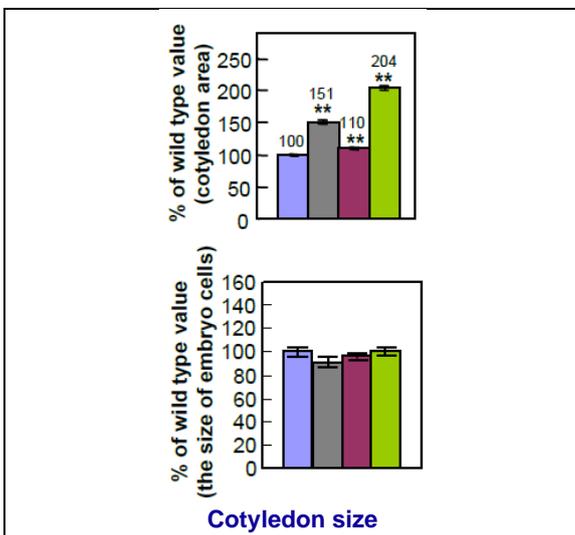
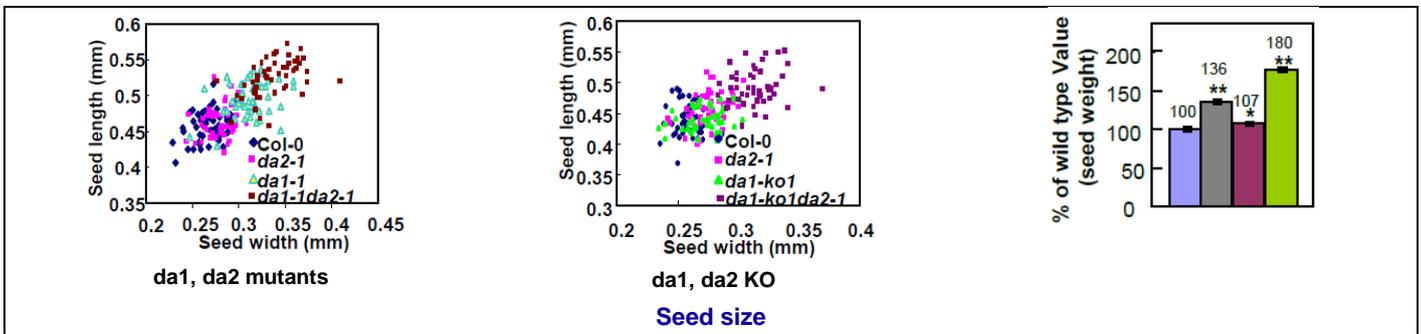
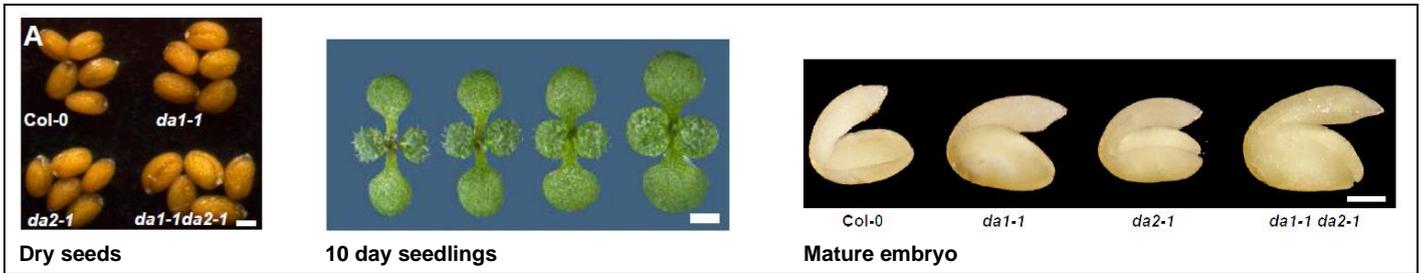


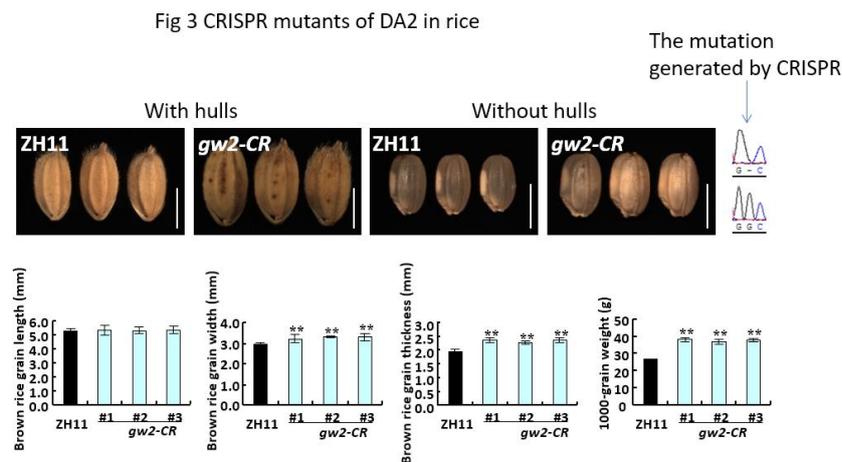
Figure 1. Seed and organ size in the *da2-1* mutant and Da2 overexpression.

Figure 2. DA1 and DA2 act synergistically to control seed and organ size, through effects on cell number.



UPDATE May 2016

The inventors at IGDB have now created CRISPR/Cas mutants in DA2 in rice (Fig 3). These lines have increased grain size and are currently being further tested and characterised, and combined with da1 mutants.



Yunhai Li, May 2016 – CONFIDENTIAL – PBL Tech Id 13.561

Rights are still available in certain plant species. For more information or licensing interest, please contact PBL

References:

Tian Xia et al. The ubiquitin receptor DA1 interacts with the E3 ubiquitin ligase DA2 to control seed and organ size in *Arabidopsis thaliana*. Plant Cell September 2013; 25 (9): 3347-3359.