A novel viviparous mutant *(vip)*

Yaxley, J.L., Weller, J.L. and Reid, J.B. ,
Department of Plant Science, University of Tasmania
GPO Box 252-55, Hobart, Tasmania 7001, Australia

We have isolated two allelic viviparous mutants of *Pisum sativum* L. Mutant seeds germinate precociously in the pod (Figs 1 and 2). These are the first mutants of this type described in the garden pea and the gene symbol *vip* has been assigned to the locus.

Abscisic acid (ABA) plays an important role in the prevention of precocious germination in many species. There are well known viviparous mutants of maize (7), caused by either impaired ABA biosynthesis (6), or decreased ABA sensitivity (8). In *Arabidopsis,* the double mutant *(aba-1 abi3)* of the ABA synthesis mutant *aba-1* and the ABA insensitive mutant *abi3,* shows viviparous germination inside the siliques (5). In species which exhibit vivipary as part of their normal development, ABA responses have also been shown to be affected. In the case of the mangrove *Rhizophora mangle* L., unusually high concentrations of ABA are required to inhibit the growth of excised embryo-seedlings (9). Embryos of most species display the ability to germinate precociously on culture media when removed from the ovule after pattern formation is complete. Such precocious germination can be prevented in many cases by adding ABA to the culture media (2,4). However, ABA may not have this role in pea. The pea ABA synthesis mutant *wil* does not exhibit vivipary, despite having one fifth the ABA level of wildtype seeds (3). In addition, the precocious germination of pea embryos in culture cannot be fully inhibited by ABA (1). Thus, this new viviparous mutant represents an important tool for greater understanding of the involvement of ABA in the precocious germination of peas, in particular, and of the switch between the seed maturation and germination programmes in general.

In a mutagenesis programme, cv. Torsdag (Hobart line 107) was treated with the alkylating agent EMS (ethylmethanesulphonate) at 1% for 6 h at 18°C. Among 1100 M_2 families screened, two showed segregation for viviparous seed. Dry *vip* seeds are not viable, so the two mutants were recovered via M_2 heterozygous siblings, as these selfed heterozygous siblings again produced viviparous seeds in the M_3. The two original mutant lines were designated as A303 and A353. These lines were crossed to each other and the F_1 seeds were viviparous, indicating that both mutations are at the same locus. We named these mutant alleles *vip-1* (A303) and *vip-2* (A353), respectively.

The *vip-1* and *vip-2* mutant seeds germinated precociously in the pod on the mother plant 20 to 25 days after opening of the flower, depending on the season (Fig. 2). This is shortly after the seed reached contact point (the point at which all liquid endosperm has been absorbed). At first, the radicle emerged and the testa split. As growth continued, the radicle extended and in the most pronounced examples (approximately 10% of a *vip* seed population) the plumule and first scale leaves also emerged from the cotyledons. Typically, the radicle reached 10 to 20 mm in length before dehydration, while the plumule and hypocotyl, if emergent, typically extended 5 to 7 mm. The morphology of the *vip* seeds did not appear to be affected by temperature or photoperiod on this background (cv. Torsdag).

Correspondence to: Jenny Yaxley Fax: 61 (03) 62 26 2698
Email: jennyy@plant.utas.edu.au

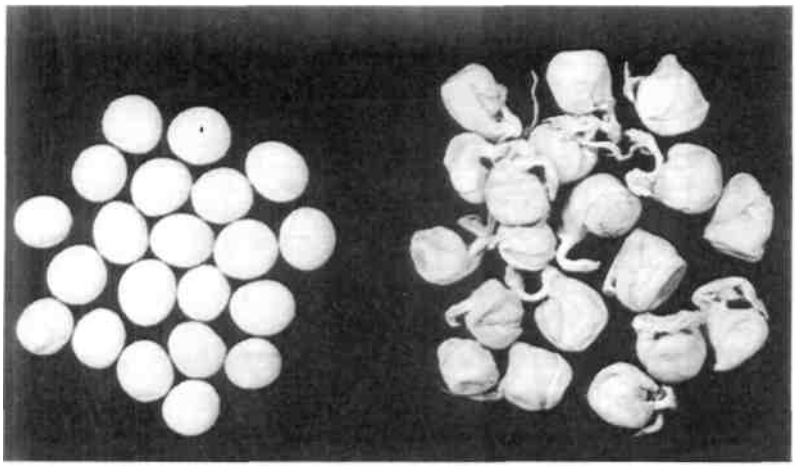

Fig. 1. Dry seeds of the precociously germinating *vip-1* mutant (right) and its wildtype progenitor cv.Torsdag (left). The viviparous seeds do not tolerate desiccation and dry *vip* seed is not viable.

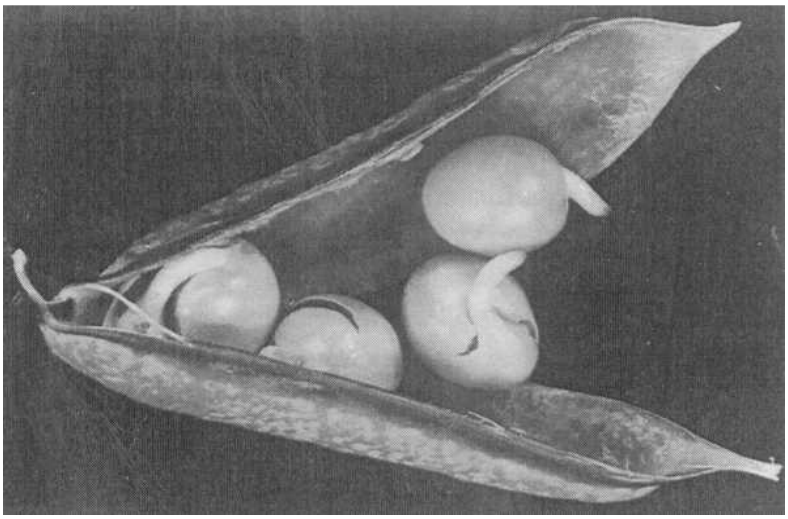

Fig. 2. Seeds of the *vip-1* mutant germinate precociously in the pod shortly after contact point is reached.

The *vip* mutant seeds remain green as maturation progresses, while the wildtype seeds turn yellow [Torsdag has yellow (*I*) cotyledons] and dehydrate. If *vip* seeds remain in the pod on the mother plant, they die through desiccation as the plant senesces. Yet, if they are removed from the pod and planted before they dry out, they will continue to grow and will generate a normal healthy plant (Fig. 3). However, *vip* seedlings have reduced early growth and survival. When they emerge from the soil they are somewhat weak and slow-growing compared with wildtype seedlings, and the axillary buds in the cotyledons and first nodes often grow out (Fig. 4). In our studies 10 to 25% of *vip* seedlings failed to survive. Nonetheless, surviving vip seedlings gradually regained vigour until their appearance was equivalent to that of the wildtype plants. Thus, it seems that the *vip* mutation is entirely seed specific, although the disraption of seed metabolism that occurs during precocious germination does have a detrimental effect on early seedling growth. The extent of seedling mortality varied with the time at which the seeds were removed from the pod, and with the growing conditions. Survival was highest when *vip* seeds were removed from a pod which was dehydrating; that is, the pod was mostly brown, but retained some green colouring, particularly along the suture. Growth and survival of the planted germinating *vip* seeds is promoted by keeping them moist, as they are sensitive to dehydration, but not too wet, as they are also prone to rotting.

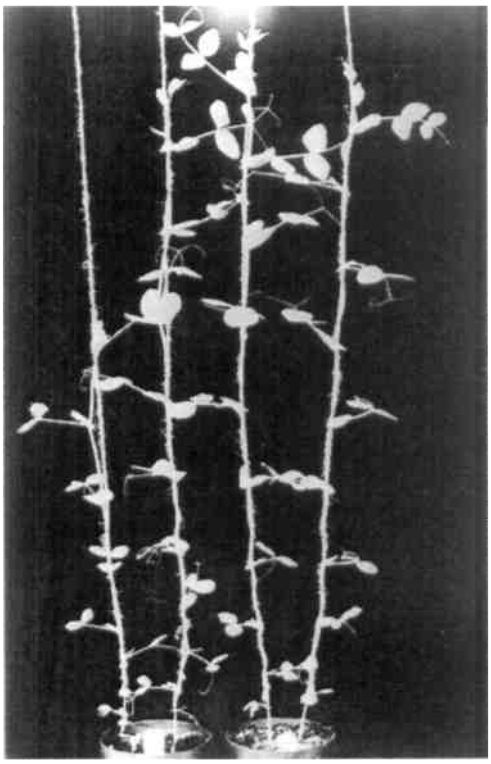

Fig. 3. Six-week-old plants of the *vip-1* mutant (left pot) and its wildtype progenitor cv. Torsdag (right pot). After an initial lag period, *vip* mutant plants have normal wildtype growth.

Fig. 4. Two-week-old seedlings of *vip-1* (right pot) and its wildtype progenitor cv. Torsdag (left pot). The *vip* seedlings have reduced early growth and survival.

There is little difference in the morphology of the *vip-1* and *vip-2* mutants. The *vip-2* mutant has better early seedling survival and growth. In some cases the *vip-1* mutant showed up to 25% seedling mortality, compared with an average of 10% for the *vip-2* mutant. For this reason, we suggest that *vip-1* is the more severe allele, causing a greater disruption of seed metabolism. In some crosses, segregation for wildtype and *vip* seeds was in accordance with a 3:1 ratio, but the combined data from several crosses indicated a significant and consistent deficiency of *vip* segregants (Table 1). There was no evidence of heterogeneity among the crosses and both alleles gave similar results with an average of 15 to 16% of *vip* seeds instead of the expected 25% (Table 1). There did not appear to be an increased number of aborted seeds in these F_2 populations, which suggests that *vip* gametes may have reduced viability. In crossing the *vip* mutants with wildtype lines, it was also determined that it is the genotype of the embryo, independent of that of the seed coat and pod, that determines the expression of vivipary. Mutant *vip* seeds showed vivipary even when contained in heterozygous *Vip vip* pods, while heterozygous seeds showed no evidence of vivipary within homozygous *vip* mutant pods. Therefore, the *vip* mutants must be deficient in a single embryo-specific factor that acts, in the wildtype, to prevent precocious germination.

Seed fresh weight, dry weight and water content for the *vip-2* mutant and wildtype were compared (Fig. 5). Seed weights were measured at the time of harvest, and then again after freeze-drying to obtain dry weight and hence water content values. The fresh weight of wildtype and *vip-2* mutant seed was similar. However, the dry weight of the *vip-2* seeds was approximately 50 mg (about 20%) less than the wildtype seeds from 15 days after flowering. In this experiment, vivipary occurred at around 20 days, so apparently the *vip-2* mutation has

Table 1. Combined data from the F_2 of several crosses for segregation of wildtype and *vip* seeds.

Segregating alleles	Phenotype *Vip*	*vip*	Total	Percentage of mutant seeds	Segregation Prob. χ^2(3:1)		Hetero-geneity χ^2(df=12)	Prob.
Vip/vip-1	253	46	299	15.4	14.74	<0.001	2.82	>0.9
Vip/vip-2	231	43	274	15.7	12.66	<0.001	4.88	>0.9

an effect on seed metabolism before the onset of vivipary, and before contact point is reached. The water content of the seed was concomitantly increased in the *vip-2* mutant, in particular at 15 days after anthesis, when the *vip-2* mutant seeds had a 13% greater water content than the wildtype seeds.

Although the appearance of vivipary is the first readily visible aspect of the *vip* mutant phenotype, it is apparent that the *vip* mutation also alters seed metabolism during earlier seed development as the seed matures and accumulates storage products. Thus, if *vip* is deficient in a factor responsible for the prevention of precocious germination, then this factor must become active during early maturation of the seed. It cannot be ruled out that the *vip* mutation affects an earlier seed-specific step which is necessary for the production of such a factor. However, as the viviparously germinating seed is still able to survive if planted out, the disruption of other aspects of seed metabolism must not be profound. An analysis of ABA metabolism and the expression of seed storage genes in the *vip* mutant will be reported elsewhere, and a linkage analysis is in progress. Pea is an excellent model species in which to examine seed development, and the isolation of this new type of maturation-germination mutant in pea will facilitate a greater understanding of these processes.

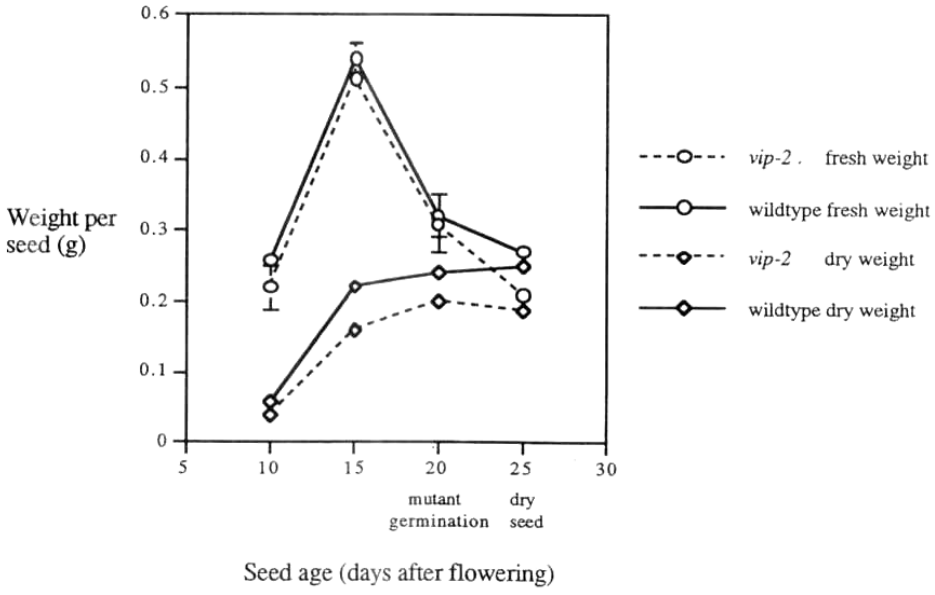

Fig. 5. Changes in fresh weight and dry weight during development of seeds of the *vip-2* mutant and its wildtype progenitor cv. Torsdag.

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