Hormone level and sensitivity mutants of pea perform as well as wild types in tissue culture

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A vast body of literature on the growth and development of pea exists, much of it derived from the study of mutants. The recent development of a reproducible system for pea transformation (12) makes it possible to further enhance physiological studies by the production of pea transformants expressing genes of interest in biosynthetic or developmental pathways. A critical part of plant transformation is an efficient protocol for tissue culture and regeneration. Interest in transforming various hormone mutants of pea with the genes for auxin biosynthesis from *Agrobacterium tumefaciens* to study the interactions of auxin and gibberellin, led us to screen various tissue culture methods for their efficacy on diverse genotypes. Jackson and Hobbs (3) reported a culture method successful with a wide range of pea genotypes including commercial varieties and wild accessions. This method was tested here on pea genotypes having altered endogenous hormone content and sensitivity, both of which may be expected to affect regenerability. The differential performance of sunflower cultures taken from various genotypes (9) and parts of the plant (11) was attributed to differing endogenous hormone levels in the explants. In lines of corn having different abilities to regenerate shoots from callus culture, it was found that high auxin levels in the starting tissue correlated with reduced competency to regenerate shoots from callus (1). These workers concluded that endogenous hormone content should be considered when selecting genotypes or tissue explants for tissue culture and regeneration. We asked here whether differences in hormonal level in our pea lines would affect their performance in tissue culture.

Methods

The commercial pea variety Progress #9 and the hormone mutants made available by Prof. J.B. Reid at the University of Tasmania, L203, L205^{-}, L205^{+}, L188, K511, WL1766 and WL5862, were used. Genotype and hormone status of these lines is shown in Table 1. Seeds were surface sterilized in 70% EtOH for 2 minutes, rinsed in sterile water, soaked in 20% chlorox for 20 minutes, rinsed four times in sterile, distilled water, and germinated aseptically in the dark at room temperature on filter paper moistened with water for three to five days. The cotyledonary nodes, consisting of 1-2 mm of tissue with the radicle and epicotyl removed, were dissected out and placed on media (Murashige Skoog basal salts, Gamborg's B5 vitamins, 3% sucrose, 0.7% agar, and either 2.5 or 5 mg/L benzylaminopurine, pH 5.7) in a growth chamber at 25° C with 16 h of fluorescent light and 8 h of dark (all chemicals and growth media obtained from Sigma Chemical Co., St. Louis MO). In some trials, the axillary bud area of the cotyledonary node was scraped to disrupt mature, preformed buds. Rooting of shoots derived from culture was done by dipping the shoot base in 1 mg/mL IAA and placing on hormone free media. Rooted plantlets were transferred to Magenta boxes (Magenta, Chicago IL) containing sterile Cornell Mix moistened with Murashige Skoog basal nutrient solution. The lids of the boxes were opened gradually over one week and finally removed to allow the plants to adjust to ambient humidity. Plants were transplanted to pots in a growth chamber.

Table 1. Endogenous hormone levels and phenotypes of the pea lines used in this study.

Line	Genotype	Phenotype	Endogenous hormone content
WL1766	*na*	Ultradwarf	GA_1 lacking (7), very low IAA (4)
L188	*le la cry^s na*	Slender	GA_1 lacking (2), very high IAA (4)
L203	*le*	Dwarf	Low GA_1 (10), low IAA (4)
L205$^-$	*le*	Dwarf	Low GA_1, low IAA
L205$^+$	*Le*	Tall (wild type)	High GA_1 (10), high IAA (4)
WL5862	*lkb*	GA-insensitive dwarf	Near WT GA_1 level (5), low IAA (6)
K511	*lh*	Dwarf	Low GA_1 (8), IAA undetermined

Results

Nine days after dissection, axillary buds on unscraped cotyledonary node explants were well developed and about 4 mm long. Two or three smaller, peripheral shoots were also observed at this time. Scraping of the node at the time of dissection successfully disrupted the axillary buds, so that only the later developing peripheral shoots emerged. The first shoots were ready for rooting (greater than 1.5 cm long) after 6 weeks. All genotypes had a uniformly high frequency, almost 100%, of explants producing shoots. Explants produced 20 to 30 shoots each, and this number did not vary substantially from WL1766, the ultradwarf, to L205$^+$, a wild type.

We found that a higher frequency of longer, more highly branched roots was achieved with an IAA dip of the shoot and growth in hormone-free medium than by transplanting shoots to media with 0.186 mg/L NAA (3).

Plantlets from tall and dwarf varieties were transplanted to soil mix in a growth chamber and there flowered, produced seed, and senesced when they had 6 to 14 internodes. These regenerants had shorter internodes and produced fewer pods with fewer seeds per pod than did plants of the same genotype raised from seed. However, seed produced from regenerants was fertile and gave rise to phenotypically normal progeny when grown in the greenhouse.

Discussion

In a range of pea mutants differing from the wild type in endogenous hormone level, the ability to regenerate shoots and roots in culture was not adversely affected. Using cotyledonary node explants, multiple shoots were produced from cultures of tall, dwarf, GA-insensitive, and ultradwarf lines of pea. These shoots represent regeneration both from immature buds and from undifferentiated cells, as the preformed axillary buds of the cotyledonary nodes were destroyed by scraping the explants. Each line retained its characteristic phenotype in culture: shoots regenerated from WL1766 explants were severely dwarfed relative to the other cultures and were dark green, while the cultures of L205$^+$ produced taller, lighter green shoots, and the WL5862 regenerants showed relatively smaller leaves and thickened stems. Because all of these hormone mutants are easily regenerated from tissue culture, the possibility is open for using a gene of developmental interest to produce a series of pea transformants having varying backgrounds of endogenous hormone level. In addition, questions on the role of endogenous hormone in the control of shoot initiation from cultured explants remain. It appears that shoot formation in pea cultures is independent of endogenous hormone level.

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