Rooting in Transgenic Peas

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INTRODUCTION

Grain legumes have generally been difficult to regenerate and transform. Of the major grain legume crops—soybean, chickpeas, peas, cowpea, peanut, common bean, faba bean, and lentils—confirmed transgenic plants have been produced in all except faba bean, lentil, and cowpea (for a review see Christou, 1994). In soybean, chickpeas, and peas, plants have been produced using A*grobacterium*-mediated transformation. For soybeans the method was unrepeatable, and for chickpeas inheritance of the transgenes was not demonstrated. For peas, there are four published reports of *Agrobacterium*-mediated transformation (Puonti-Kaerlas et al., 1990; Schroeder et al.,1993; Davies et al., 1993; Grant et al., 1995). The Puonti-Kaerlas method takes 15 months to recover transgenic plants, all confirmed as tetraploid. While the method of Schroeder is successful in their hands, others have been unable to repeat it (A. de Kathen, University of Hanover and C.M. Stiff, University of Washington, pers. comm.). Davies developed an injection method that is not reproducible even in their own laboratory (D.R. Davies. John Innes Centre, Norwich, England pers. comm.).

At Lincoln we have developed a reliable method to produce transformed plants in a wide range of arable and processing pea cultivars. The explants used are immature cotyledons from "eating" stage peas from either field-grown or glasshouse-grown plants. The peas are at the stage of maximum size and before bleaching of the chlorophyll and drying of the seed. They are dissected, and the proximal half of the cotyledon and the embryo are discarded. The remaining half of the cotyledon is immersed for 1 h in an overnight culture of *Agrobacterium tumefaciens*. After co-cultivation the explants are grown on a medium containing a selection agent.

Callus forms on the cotyledon attachment scar and from this callus, transformed shoots are regenerated. The first transformed shoots are recovered after approximately 4 months and keep producing new transformed shoots for another 5 months. The first shoots root easily using 2 mg litre^{-1} IBA (indole-3-butyric acid)as described by Grant et al. (1995), however, after about 6 months in culture the rooting efficiency decreases. As we wish to maintain shoot cultures of transformed lines for micropropagation and as backup cultures, successful rooting of older material is important. Micropropagation of the transformed shoots and their subsequent growth in the glasshouse allows us to collect a larger number of seeds from the primary transformant, than would otherwise be available, to form the next generation for testing. In this paper we describe experiments carried out in an attempt to improve the rooting of transgenic pea shoots that have been in tissue culture for extended periods.

MATERIALS AND METHODS

Transgenic pea shoots, from eight cultivars and breeding lines of arable and process peas that had been co-cultivated with a range of vectors, were used for the two rooting experiments. For each treatment the shoots were visually graded for size so that similar shoots could be randomly placed into each treatment. In the first experiment 77 to 79 shoots per treatment were used, and in the second experiment there were 41 to 43 shoots per treatment.

For the treatments with the auxin in agar, the basic medium was Gamborg's B5 (1986) containing 30 g litre^{-1} sucrose, 8 g litre^{-1} agar (Difco), 150 mg litre^{-1} timentin (Beecham Research Laboratories), pH 5.8, to which was added one of the following: 2 mg ml^{-1} IBA, 5 mg ml^{-1} IBA, or 2 mg ml^{-1} NAA (α-naphthaleneacetic acid). Explants were cultured in these media for 6 days and then transferred to the basic medium without growth regulators.

For treatments with the auxin used as a dip, the auxin was made up in 50% ethanol and filter sterilized. The treatments were 2 mg ml^{-1} NAA, 5 mg ml^{-1} NAA, 2 mg ml^{-1} IBA, 5 mg ml^{-1} IBA, 1 mg ml^{-1} IBA + 1 mg ml^{-1} NAA, and 2.5 mg ml^{-1} IBA + 2.5 mg ml^{-1} NAA. The plantlets were given a quick dip in an auxin solution and then placed on the basic medium without growth regulators, as described above.

Plantlets were scored for rooting after a total of 4 weeks in culture. A subset of 20 shoots per treatment were transferred to the glasshouse into flats containing pure perlite, and regularly liquid-fertilised using modified Hoaglund's solution (Noggle and Fritz, 1976). The shoots were covered to maintain high humidity for 1 to 2 weeks and survival was assessed after 4 weeks.

Table 1. Rooting of transgenic pea shoots after 28 days.

Hormone treatment	Experiment 1		Experiment 2	
	Shoots rooted per treatment	Percentage rooting	Shoots rooted per treatment	Percentage rooting
2 IBA agar[1]	28/79	35%	17/43	40%
5 IBA agar[1]	43/79	54%	19/42	45%
2 NAA agar[1]	30/78	38%	15/42	36%
2 NAA dip[2]	32/78	41%	nt	nt
5 NAA dip[2]	nt	nt	22/41	54%
2 IBA dip[2]	24/77	31%	nt	nt
5 IBA dip[2]	nt	nt	18/41	44%
1 IBA/1 NAA dip[2]	33/78	42%	nt	nt
2.5 IBA/2.5 NAA dip[2]	nt	nt	18/42	43%

nt = not tested; [1] = no. mg l^{-1} in solidified media; [2]= no. mg ml^{-1} in 50% ethanol, liquid dip.

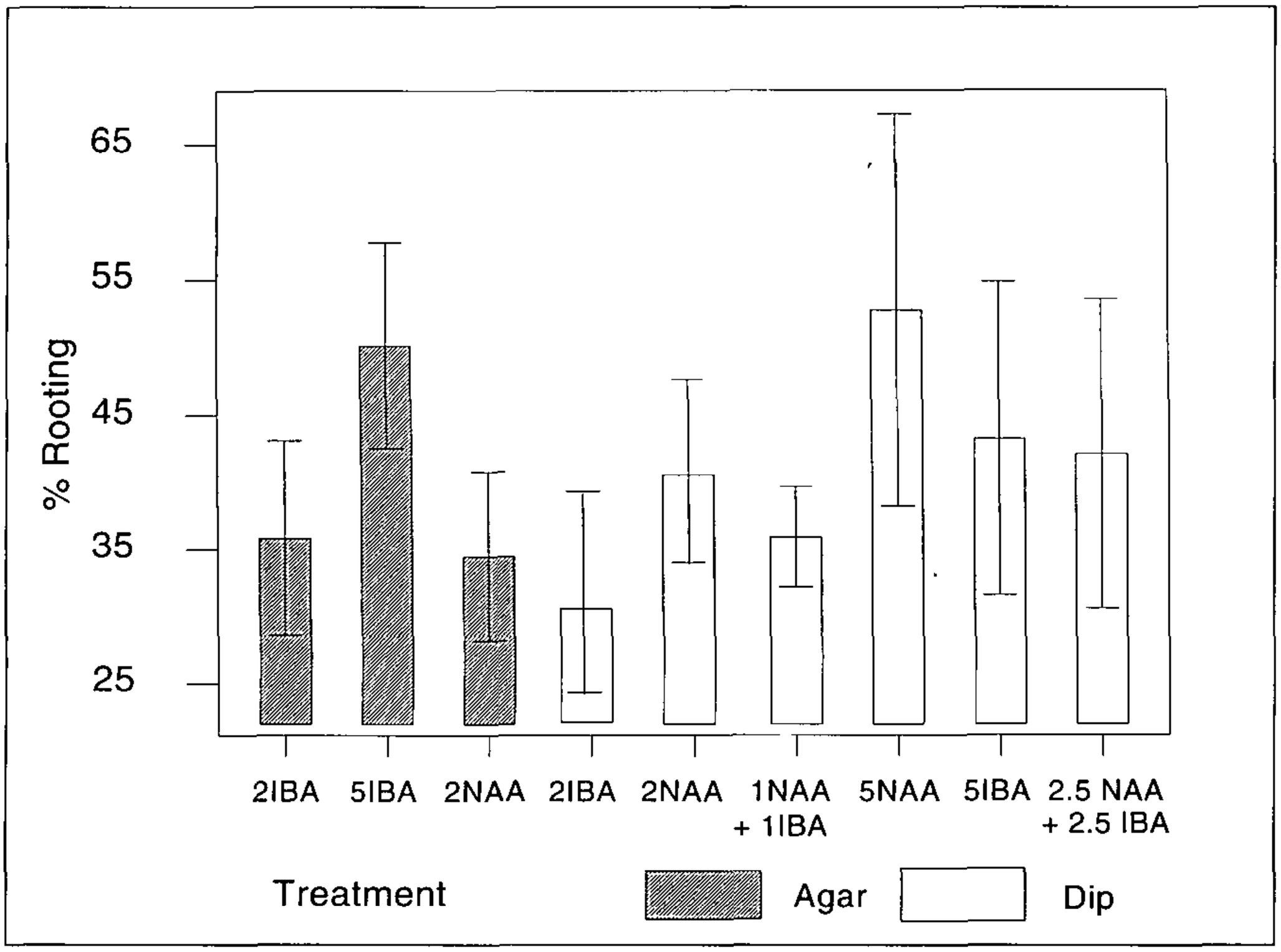

Figure 1. Rooting of transgenic pea shoots after 28 days.

RESULTS AND DISCUSSION

The results of the experiments are shown in Table 1 and Fig. 1. In Figure 1 the results of the agar treatments were pooled across the two experiments. The error bars represent one standard deviation in each direction. From Table 1 it appears that there is a small improvement in the rooting for treatments with increased auxin—5 mg ml^{-1} IBA in agar, 5 mg ml^{-1} NAA, and 5 mg ml^{-1} IBA liquid dips. The results of an analysis of variance showed no significant difference between the treatments. The variation in the results was due to the differences between each container rather than as a result of the rooting treatment. Owing to a shortage of material, some containers had shoots that were the same cultivar and others had shoots from up to three different cultivars. Pea genotypes exhibit a good deal of difference in seedling vigour in the field and this has been attributed, at least in part, to the ability to root well. Some late maturing process pea genotypes are also noted for their ability to root well and withstand "non ideal" environmental conditions better than other genotypes (D.R. Goulden. Crop and Food Research, Christchurch, N.Z., pers. comm.). Our experiments confirm such observations. Using the above treatments, there was no significant improvement in the rooting of our transgenic pea shoots overall. However, comparing the containers with high numbers of shoots that rooted, i.e. the better rooting genotypes, the treatments with higher auxin were consistently better. In the containers that had "poorer rooting" cultivars, the higher levels of auxin were always amongst the better treatments and were never the worst treatment. Further experiments using other auxins (e.g. indoleacetic acid) are underway.

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