PROGRAMMING TISSUE CULTURE-PRODUCED PLANT PERFORMANCE

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Abstract. The field performance of tissue culture-propagated strawberry plants can be manipulated by the addition of plant growth regulators in the culture medium. The addition of abscisic acid or paclobutrazol tended to produce more reproductive organs The addition of benzyladenine and gibberellins tended to produce more vegetatively vigorous plants. These results, along with data from endogenous abscisic acid determinations and light micrographs of treated meristems, further describe possible causes of *in vitro*-induced rejuvenation

REVIEW OF LITERATURE

Tissue culture (TC) propagated strawberry plants perform more like juvenile seedlings (JS) than adult plants propagated by runners (RP). Runnering is increased, flowering is delayed and susceptibility to diseases is altered (6). The cause of this phenotype is unknown. Gibberellic acid (GA) has been implicated in floral inhibition, enhanced runner development and juvenility (2), but no GA is included in any stage of strawberry *in vitro* propagation media. Benzyladenine (BA), a cytokinin included in the proliferation subcultures, has been implicated in both juvenility (3) and increased bud activity in field-grown strawberries. In a previous experiment, abscisic acid (ABA) concentrations were lower in TC-propagated and JS day-neutral strawberry plants, when compared to adult RP-plants (5). ABA is known to reduce lateral bud break and it has reduced rooting in tissue-culture strawberry explants. Therefore, reduced ABA content in TC-propagated plants would explain their increased lateral bud activity and ease of rooting. We now report three experiments where the effects of ABA, BA, GA and paclobutrazol (a GA biosynthesis inhibitor), supplied in the culture medium, are correlated with subsequent greenhouse performance of TC-propagated strawberry plants.

MATERIALS AND METHODS

'Fern' (Expts. 1 and 2) or 'Tribute' (Expt. 3) day-neutral strawberry runner meristem-tips were sterilized and subcultured on various plant growth regulators (PGR) in a Murashige and Skoog medium. Paclobutrazol and GA (both at 10 μM) were filter sterilized and added to the TC-medium after autoclaving, while BA and ABA at 5 μM and 0.5 μM, respectively, were added before

autoclaving. A no PGR control was also included in some experiments. Ten to twelve plants per treatment were established in the greenhouse and various growth variables were measured for up to 20 weeks. All measurements were analyzed by ANOVA and LSD at 5%.

Treatments for Experiment 1 included three consecutive subcultures on two levels of ABA or two levels of BA in factorial combination. Data are presented only for the treatments at 5 μM BA and 0.5 M μABA, alone and in combination (BA + ABA). Lower concentrations gave intermediate responses and no interactions occurred. Other treatments in this experiment were JS and adult RP plants. Data on number of inflorescences, runners, and branched crowns per plant at 16 weeks after propagation in the greenhouse were used to determine the relative proportion of vegetative vs. floral meristem development (Table 1).

Table 1. The effect of propagation type and *in vitro* applied plant growth regulators on the percentage meristem development at 16 weeks after propagation.

Treatment and propagation type	Percentage runners	Percentage crowns	Percentage inflorescences
Seedlings	65	25	10
TC-BA	54	29	17
TC-control	41	23	36
TC-BA + ABA	34	24	42
TC-ABA	19	21	60
Adult runners	19	20	61
LSD (5%)	10	8	7

Experiment 2 included one subculture on one PGR and a subsequent subculture on a different PGR. The PGRs used are given in Table 2. All PGR concentrations were as above. Data was cumulatively summed after 16 weeks.

Experiment 3 was a PGR X polyamine factorial in which *in vitro* treatments were applied for three subcultures and subsequent greenhouse growth was monitored for 16 weeks. ABA or BA treatments were combined with either no polyamines or filter sterilized putracine, spermadine, or spermine at 1 mM.

RESULTS

In Experiment 1, control TC-propagated strawberry plants dedicated a greater percentage of their meristems to vegetative growth (runners and branched crowns) than adult RP-plants (Table 1). In contrast, JS plants were more vegetative than TC-propagated plants. ABA acted as an anti-rejuvenation hormone while BA tended to rejuvenate plants, i.e. make them more vegetative.

Table 2. The effect of various PGR treatments in two subsequent subcultures on the number of runner plants and number of inflorescences produced by TC-propagated plants within 16 weeks *ex vitro*.

First culture PGR	Second culture PGR	Number of runners produced	Number of inflorescences produced	Inflorescence to runner ratio
BA	Control	6.7	1.8	0.27
BA	ABA	5 3	2.4	0.45
BA	GA	12.3	1 2	0 10
BA + ABA	Control	6.6	2 0	0.30
BA + ABA	ABA	7 6	2 5	0.33
BA + ABA	GA	16 8	1 5	0.09
Paclo	Control	2 3	2.3	1 00
Paclo	ABA	1 0	4 3	4.30
Paclo	GA	8 4	1 8	0.21
LSD 5%		2 1	0 9	—

In the second experiment, paclobutrazol- and ABA-treated TC-plants were more adult-like while GA increased the proportion of vegetative meristems (Table 2). A single subculture on GA increased runnering by a factor of 2 to 4. The addition of GA in the second subculture had the largest overall effect.

In Experiment 3, ABA again enhanced 'Tribute' day-neutral flowering. At 16 weeks of greenhouse growth, ABA-treated TC-produced plants produced 1.5 inflorescences per plant while BA-treated plants produced 0.1 inflorescence per plant. BA-treated TC-produced plants had only slightly more runners than ABA-treated plants over 16 weeks *ex vitro* (BA = 13.0 runners; ABA = 10.7 runners). The same treatments were applied to 'White Pine', a short-day cultivar. Flowering was not induced by ABA treatments; however, BA again slightly stimulated runnering (BA = 15.7 runners per plant; ABA = 13.7 runners per plant). Polyamines had no significant effect on plant performance.

DISCUSSION

In terms of TC-produced plant runnering and/or flowering, ABA and paclobutrazol added in tissue culture media produce more adult plant responses while GA and BA produce a response more typical of JS. Similar results were obtained in three separate experiments in our laboratory (5). In one experiment, the ABA content of TC-produced, JS, and RP plants was measured. When compared to adult RP plants, endogenous concentrations of ABA were lower in TC-propagated and JS strawberries at 3 and 7 weeks after propagation. At 15 weeks, all ABA levels were equivalent.

Based on the above information, changes in ABA physiology in TC-produced plants seems to be at least partially responsible for

their rejuvenation. ABA does not effect runnering to the extent GA and paclobutrazol do, so it is possible the GA physiology changes during *in vitro* culture as well.

The causes of these changes are unknown. ABA is thought to be synthesized or resident in chloroplasts and chloroplast function and number are reduced *in vitro*. ABA inhibitors, like fluridone and norflurazon are being tested for their effect on TC-induced rejuvenation and juvenility.

A possible explanation for the observed treatment effects is that early differences in photosynthetic rates between PGR treatments gave some plants a developmental advantage that is maintained over the study period. While ABA and paclobutrazol treatments yielded more adult plants and had higher net photosynthesis on a leaf area basis, there was no correlation between rejuvenation and photosynthesis on a whole plant basis. This contrast arises because leaves on BA- and paclobutrazol-treated plants were much smaller than GA- and ABA-treated plants (5).

In contrast, correlation between leaf form, plant behavior and meristem anatomy was found (4). The flattened apical meristems of ABA- and paclobutrazol-treated plants were typical of pre-floral differentiation stage meristems. ABA- and paclobutrazol-treated plants had more adult trifoliolate leaves (5). GA- and BA-treated plants had elongate meristems and more juvenile monofoliolate leaves. Thus, meristem pattern evidently continued for a period of time *ex vitro*. The larger response to second subculture treatments, in comparison to those in the first subculture (Table 2), could be interpreted as the last PGR which fashions *in vitro* meristem morphology will be the one controlling plant behavior. This continuation in plant developmental pattern evidently took place in TC-rejuvenated plants where endogenous ABA concentrations in the meristem are reduced. If ABA is important in developmental changes, then TC-produced plants may be matured more rapidly when exposed to conditions like short days and drought stress that increases ABA level in plants. TC-produced raspberry plants are very sensitive to short-days suggesting that they are very sensitive to increases in endogenous ABA. In addition, the water stress of acclimatization *ex vitro* temporarily raised the ABA levels of TC-produced plants (5).

Finally, it is important to realize that short-day TC-produced strawberry plants, when grown under non-inductive conditions, did not flower in response to added ABA. Short-day RP-plants also did not respond to added ABA (1). Thus, there is a level of control of flowering not affected by these hormones under the conditions of our study.

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