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Evaluation of Different Explant Sources and Growth Regulators on Callus Culture and Regeneration in Strawberry (*Fragaria ananassa*)

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Abstract: The potential of callus culture and regeneration was evaluated in these experiments. The effect of different hormonal combinations and explant sources was studied in-order-to produce maximum no. of plant in shortest possible time. Calif induced from *in vitro* grown plant exhibited high regeneration as compared to those induced from glasshouse grown plants. The effect of hormones such as BAP, IBA, NAA and 2, 4-D was studied on callus initiation and regeneration.

Key words: Callus, *in vitro,* regeneration, glasshouse

Introduction

Strawberries are traditionally propagated by means of runners and crown division. One method that has been used is micropropagation (Boxus, 1974; Boxus *et al.,* 1977; Damiano, 1980). Generally this has been considered relatively safe, although occasional morphological variants have been found (Swartz *et al.,* 1981). This methodology although attractive in comparison to traditional runner production can be unresponsive to needs. An alternative system based on the exploitation of callus and regeneration has been suggested by Nishi and Oosawa (1973). Callus is a coherent and amorphous tissue, produced on explants *in vitro* as a result of wounding and in response to hormones, either endogenous or supplied in the medium. Most workers do not advocate the use of callus as a means of regeneration/mulnolication because of the problem of variability. Indeed some phenotypic variation has been reported recently among callus culture regenerants (Jones *et al.,* 1988; Nehara *et al.,* 1990). However some species appear to produce stable callus (Hussey, 1983, 1986). For others it is thought that environmental variables during culture may influence the level of variation. However to exploit this approach will require the production of genetically stable callus. A number of variables during culture such as medium composition, number of sub-culture/generation cycle may influence the production of genetically stable callus (Shaeffer *et al.,* 1980). The main aim of this study was to evaluate the effect of different culture variables on the genetic uniformity of plants regenerated through callus culture and regeneration in strawberry (*Fragaria ananassa*).

Materials and Methods

Plant Material: Young leaves newly developing from glasshouse and *in vitro* grown strawberry plants of cuitivar Tango were harvested and surface sterilised. These leaves were used to cut the leaf discs with the help of a sterile metal cork borer with internal diameter of 6 mm.

Culture Medium: The basal medium consists of Murashige and Skoog (1962) mineral salt. This was supplemented with 3% sucrose. 0.2% phytogel and adjusted to pH. 5.7 with NaOH before autoclaving. Six media differing only in plant growth regulator type and concentration were investigated in this study as described in Table 1.

Experimental Procedure: Cali were produced by planting three sterile leaf discs in each Petri dish with 5 replications on callus initiation media (Table 1) for an initial period of 6 weeks. All the leaf discs were marked 1, 2 and 3 on the Petri dish randomly. Atter scoring the callus growth, calli were transferred to fresh media for another 4 weeks. After remaining a total of 10 weeks on callus initiation media, shoots produced on each leaf disc were counted. Regenerated plants with sufficient roots were transferred to the mixture of 5050 perlite and compost. Plants were kept in hardening conditions for four weeks and then transferred to 9 cm plastic pots containing compost and watered regularly.

The number of each category of shoots in each leaf disc were counted and data produced were subjected to analysis to investigate the statistical differences with respect to regeneration ability for the different types of media under study.

Results

Regeneration from in vitro culture leaf explants: Analysis of variance revealed highly significant differences ($p < 0.01$ and $p < 0.001$) among the media for total shoot production and number of plants survived respectively. Figure 1 shows that calli on media 3 and 5 did not produce any shoots while the maximum number of shoots were produced on medium 29 containing BAP/IBA 2.25/1 mg/l. There was good shoot production on medium 28 but this medium produced 64% abnormal shoots as well Medium 29 produced the maximum number of plants that survived. There was apparently no difference in media 10, 13 and 28 on plant survival (Fig. 2). Medium 28 produced more total number of shoots as compared with media 10 and 13, but abnormal shoots on medium 28 failed to survive when transferred to rooting media.

Regeneration from glasshouse-grown leaf explants: The leaf discs were taken from leaves harvested from glasshouse grown plants. All the other experimental conditions and methodology were exactly similar to the previous experiment described above. Data was collected after 10 weeks and subjected to analysis of variance to investigate the statistical difference for regeneration ability for the different types of media (Table 1 if under study).

Statistical analysis revealed highly significant differences among the media for total shoot production and number of plant survived for both ($p < 0.01$). Figure 3 indicates that maximum number of shoots were produced on medium 13 whereas medium 10 produced all abnormal shoots, It was also indicated (Fig. 4) that the maximum number of

Table 1: Combinations and concentration of different growth regulatoers added to culture media

Media	Cytokinin	Concentration	Auxin	Concentration
Media-3	BAP	1.12 mg/l	2, 4.0	1.1 mg/l
Media-5	BAP	2.25 mg/l	2, 4-0	0.22 mg/l
Media-10	BAP	1.12 mg/l	NAA	0.186 mg/l
Media-13	BAP	2.25 mg/l	NAA	0.186 mg/l
Media-28	BAP	2.25 mg/l	IBA	0.2 mg/l
Media-29	BAP	2.25 mg/l	IBA	1.0 mg/l

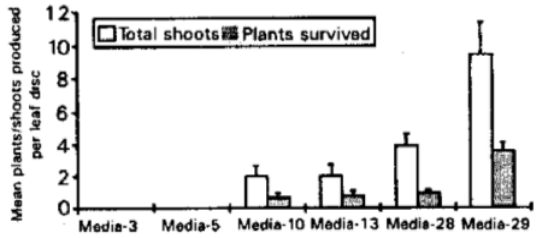

Fig. 1: Mean number of shoots/plants survived produced from each leaf disc *in-vitro* grown leaf explant on different media

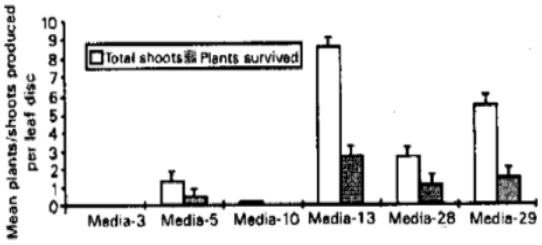

Fig . 2: Mean number of shoots/plants survived produced from each leaf disc derived from glasshouse-grown leaf explant on different media

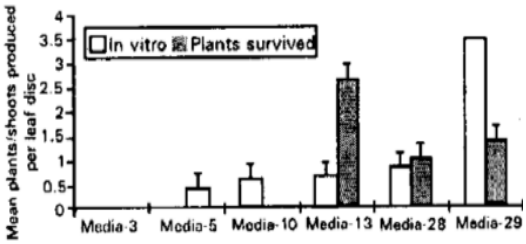

Fig. 3: Effect of leaf explant source environment on the production of number of plants on different media

plants that survived were found on medium 13, while medium 29 produces second highest.

Figure 3 compares the two types of explant sources, indicating the influence on the same genotype/cultivar of different environment growth conditions. Leaf discs taken from *in vitro* culture explants source produced maximum plants on medium 29 whereas leaf discs taken from glasshouse grown plants produced maximum plants on medium 13. In contrast to leaf discs taken from glasshouse grown plants, the leaf discs taken from *in-vitro* grown explant did not produce any plants on medium 5 but it produced some plants on medium 10.

Discussion

Various combinations of cytokinin (BAP) and auxins (2,4-D, NAA, IBA) were tested in preliminary experiments. BAP 2.25 mg/l in combination with 0.18 and 1.0 mg/l with NAA and IBA respectively gave maximum shoots regeneration. Media containing 2,4-D produced only callus at 1/1 ratio with BAP while at higher BAPI2,4-D ratio produced a substantial number of shoots in glasshouse-grown leaf explants only (less than 0.5 per leaf disc).

The organogenic potential of explants from *in vitro* culture shoots compared with those taken from glasshouse-grown plants was significantly different for the same media. In general, the calli from *in vitro* leaf explants exhibited higher regeneration frequency than those induced from glasshouse-grown. These results are similar to those obtained in various other studies by Liu and Sanford (1988) and Jones *et al.* (1988), for strawberry and Cousineau and Donnelly (1991), in raspberry. These workers found that different concentrations of growth regulators were necessary to optimise regeneration from both types of explants. In the *in vitro* explants the high regeneration frequency may be due to their adaptation to *in vitro* environments. Maximum shoot regeneration from *in vitro* was found on regeneration media-29 containing BAP/IBA 2.25/1.0 mg/l whereas, glasshouse-grown explants gave maximum shoots on medium 13 containing BAP/NAA 2.25/0.1 mg/l. The *in vitro* shoots in this study were maintained on BAPABA 1.0/1.0 mg/l prior to explant preparation. Thus, the poor shoot regeneration on leaf explant taken from *in vitro* shoots may possibly *be* due to a hormonal effect between NAA and IBA interaction caused by accumulation of IBA in the leaf tissue.

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