A one step in vitro cloning procedure for Red Globe grape: The influence of basal media and plant growth regulators

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Abstract

Earlier studies have shown that the degree of success at each stage of micropropagation in grapevine is genotype dependent; hence it becomes imperative to optimize culture conditions for rapid propagation of a variety. Present report describes two approaches of in vitro propagation of a Vitis vinifera cultivar, Red Globe. In one approach, whole plants could be developed from single node segments by bud break and direct rooting in vitro. Eight different basal media tried showed different morphogenetic responses. In second approach, multiple shoots were induced in nodal segments cultured on MS basal medium supplemented with BA (8.88 µM). Also, second crop of shoots could be induced in left over nodal segments devoid of shoots. Rooting of shoots could be induced in vitro, both in semi-solid or liquid media and also ex vitro by pulse treatment of IAA (2.85 µM) + NAA (2.70 µM). Plant establishment in later case was 80%. A simple procedure described here can complement conventional methods, currently being used in propagation of this important grape variety.

Key words: Auxin pulse, benzyladenine, grape, micropropagation, Red Globe, Vitis vinifera

Introduction

Due to heterozygous nature grape varieties are mostly propagated by vegetative means. Application of plant tissue culture techniques in propagation and improvement of grapevines has been reviewed by several workers (Krul and Mowbray, 1984; Gray and Meredith, 1992; Torregrosa et al., 2001). The technique has been used to propagate pathogen free grapevine stock (Duran-Vila et al., 1988). Micropropagation complements the conventional technique when a large number of propagules of a particular variety are required in a shorter time. Earlier studies on in vitro propagation of Vitis have indicated that the degree of success at each stage of culture is genotype dependent and varies under a given set of culture conditions (Barlass and Skene, 1980; Monette, 1988; Botti et al., 1993). Hence, it becomes essential to optimize culture conditions for a particular clone / cultivar / rootstock or newly bred line that needs large scale planting but availability of sufficient planting stock is a limitation. The present communication describes influence of eight basal media and growth regulators on micropropagation of Red Globe, a Vitis vinifera cultivar. The variety is in great demand due to its attractive reddish-purple colour; taste bud arousing flavour and appealing large plum-size berries with uniform bunches.

Material and methods

Twigs of field grown, disease free vines of Red Globe were collected from the vineyard of National Research Centre for Grapes, Pune. These were defoliated and cut into single node segments (2 cm). The explants were dipped in 1% Labolene solution for 10 min; rinsed with tap water; submerged in 0.1% Bavistin solution; kept on a shaker (120 rpm) for 2 h and thereafter rinsed three times with sterile water in a laminar flow hood. These were then disinfected with 0.1% mercuric chloride solution for 10 min and rinsed three times with sterile water. The explants were finally blotted dry on sterile filter paper and inoculated on medium in glass test tubes (150 X 25 mm).

For budbreak, eight different basal media – MS (Murashige and Skoog, 1962), WPM (Lloyd and McCown, 1980), NN (Nitsch and Nitsch, 1969), B5 (Gamborg et al., 1968), ER (Eriksson, 1965), LS (Linsmaier and Skoog, 1965), Cd (Chee and Pool, 1987) and GNMG (Galzy et al., 1990) devoid of growth regulators were tested. Another experiment with MS medium and range of BA concentrations (0.04 to 11.1 µM) was undertaken to maximize budbreak. To obtain second crop of shoots, primary nodal segments left after excising the grown axillary shoot (hereinafter referred to as mother explant) instead of its discard, were transferred to WPM or MS with or without BA (4.44 and 8.88 µM).

For induction of multiple shoots, axillary shoots obtained from primary nodal segments were inoculated (S0) in test tubes having MS medium with BA (2.22 to 8.88 µM). After 30 d, explants showing multiple shoots were transferred to fresh medium in glass bottles. This was continued at an interval of 30 d until five transfers (from S1 to S5). To test the effect of inoculum’s density per culture vessel, two, three, four or five shoot clumps per culture bottle were inoculated on MS with BA at 4.44 or 8.88 µM. Two sets of experiments were carried out for elongation of in vitro shoots. In the first set, shoots less than 3 cm in length were inoculated on WPM supplemented with or without BA (2.22-8.88 µM). In the second set, multiple shoot clumps with shoots of <1.5 cm in length were kept for elongation. These were inoculated in glass bottles containing MS supplemented with BA (2.22 or 4.44 µM) and NAA (0.54 µM).

For in vitro rooting, shoots more than 3 cm were inoculated in test tubes containing half or full strength MS or WPM supplemented
with NAA (0.54 - 1.07 µM) or IAA (0.57 - 1.14 µM) or IBA (0.49 - 0.98 µM) or IPA (0.53 - 1.06 µM) with or without agar. Liquid media had filter paper bridges. In vitro raised shoots, more than 7 cm in length were given pulse treatment of different auxins, IAA (2.85 - 5.71 µM) or IBA (2.46 - 4.90 µM) or IPA (2.64 - 5.29 µM) or NAA (2.69 - 5.37 µM) either singly or in combination for 10 min and then planted in plastic cups containing a mixture of coco-peat + soil + sand (1:1:1). Untreated shoots served as control. Shoots rooted were taken out of the culture tubes, their roots gently washed with water to remove adhering medium and were transferred to plastic cups containing the above mentioned mixture. Plants were acclimatized by the Sachet technique (Ravindra and Thomas, 1985). Plants after transfer to cups were kept in continuous light of 24.4 µmol m$^{-2}$s$^{-1}$ at 25 ± 2°C. Thereafter, these were shifted to another growth room at ambient temperature (35 ± 2°C). Establishment of plants was recorded after 30 d.

All the media were supplemented with sucrose 20 g L$^{-1}$ and gelled with agar 7 g L$^{-1}$. The pH of the media was adjusted to 5.8 before autoclaving at 121°C for 20 min. Cultures were incubated under 16 h photoperiod obtained with cool light fluorescent tubes with light intensity of 24.4 µmol m$^{-2}$s$^{-1}$ at 25 ± 2°C. Experiments were repeated at least three times. Observations were recorded at monthly interval. The experiments were conducted in Completely Randomized Design and the results were subjected to analysis of variance.

### Results and discussion

Bud break in nodal segments commenced from the fifth day of inoculation and continued up to 20$^{th}$ day and thereafter shoots put forth rapid growth. Among the eight basal media tested, C$_{d}$, LS and WPM without any growth regulators induced 92, 90 and 84% budbreak, respectively. Induction of two or more shoots in maximum explants (76%) was observed in C$_{d}$ medium. NN induced the minimum response (Table 1). In addition to budbreak, nodal explants both in WPM and B5 induced rooting at the base in 70% of explants. In case of NN, it was 46%. In all other media rooting was very low. These rooted nodal segments with primary shoots could be established on potting and were hardened by the Sachet technique. Thus, no special difficulty was faced with nodal culture producing entire plantlet.

Eight different nutrient media induced different morphogenetic responses in nodal segments. Shoots in C$_{d}$ were found to be stunted, succulent with light green, thick leaves and glossy in appearance. Basal media LS and Eriksson showed necrosis in shoot tip, which continued to the entire shoot and caused drying of the whole shoot. Shoots in NN lacked vigour, had thin stems with dark green leaves. MS resulted into comparatively better shoots with normal internode and light green leaves. Also, shoots on MS were most vigourous as compared to other media tested. The shoots in B5 were similar to those observed in MS except that the internode was slightly thicker. The shoots in WPM lacked vigour and had thin, lanky stems showing twining habit with thin foliage. Of the eight media tested, MS was found to be the most suitable medium resulting into vigorous shoots. Hence, for multiple shoot induction experiment, only MS was used.

In a similar study on basal medium, Reisch (1986) observed significant differences in growth in grape cultivar White Riesling with MS half and MS full medium. However, in contrast to the present study, Gray and Benton (1991) observed stunted growth in shoots of Muscadine grape cultivars when WPM was used. Genotypic variability within Vitis vinifera cultivars cultured in vitro has earlier been reported (Harris and Stevenson, 1982; Chee and Pool, 1983; Galzy et al., 1990). Varying response of different genotypes to different basal media could be due to variations in nutrient compositions. For example, amount of CaCl$_2$ is higher in MS, LS and Eriksson as compared to WPM and NN, while in C$_{d}$ and GNMG, it is substituted by Ca(NO$_3$)$_2$. Similarly, Potassium Iodide (KI) is absent in WPM, NN, C$_{d}$ and Eriksson while it is present in GNMG, B5, LS and MS though in different quantities. Also amounts of MnSO$_4$ vary in the eight basal media tested.

Galzy (1969) demonstrated that mineral requirement varied with the morphogenic process: strong K and N concentrations proved favorable to shoot development but impeded root growth. Chee and Pool (1987) working with grape tissues have reported that lower concentrations of KI and MnSO$_4$ in the medium were good for maximum shoot production and incorporation of Ca(NO$_3$)$_2$ instead of CaCl$_2$ produced shoots of good quality. Present study corroborates these findings since maximum results of budbreak of shoots were obtained from explants inoculated on C$_{d}$ though shoots obtained from explants inoculated on MS medium were comparatively healthy and vigorous. Besides nutrients, differences in in vitro response between genotypes of different species may be related to differences in endogenous

<table>
<thead>
<tr>
<th>Basal medium</th>
<th>Explants showing bud-break* (%)</th>
<th>Explants showing single shoots (%)</th>
<th>Explants showing 2 or more shoots (%)</th>
<th>Total number of shoots obtained</th>
<th>Soots elongated (%)</th>
<th>Average shoot height (cm) ± S.D.</th>
<th>Explants showing rooting at the base (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NN</td>
<td>42</td>
<td>38</td>
<td>04</td>
<td>23</td>
<td>82.6</td>
<td>2.13 ± 0.10</td>
<td>46</td>
</tr>
<tr>
<td>C$_{d}$</td>
<td>92</td>
<td>16</td>
<td>76</td>
<td>97</td>
<td>44.0</td>
<td>3.52 ± 0.46</td>
<td>02</td>
</tr>
<tr>
<td>B5</td>
<td>70</td>
<td>32</td>
<td>38</td>
<td>58</td>
<td>52.0</td>
<td>3.90 ± 0.23</td>
<td>70</td>
</tr>
<tr>
<td>MS</td>
<td>80</td>
<td>18</td>
<td>62</td>
<td>96</td>
<td>37.0</td>
<td>1.86 ± 0.35</td>
<td>10</td>
</tr>
<tr>
<td>LS</td>
<td>90</td>
<td>24</td>
<td>66</td>
<td>98</td>
<td>38.0</td>
<td>1.82 ± 0.36</td>
<td>14</td>
</tr>
<tr>
<td>ER</td>
<td>68</td>
<td>04</td>
<td>64</td>
<td>83</td>
<td>39.0</td>
<td>1.19 ± 0.10</td>
<td>04</td>
</tr>
<tr>
<td>WPM</td>
<td>84</td>
<td>40</td>
<td>44</td>
<td>68</td>
<td>44.0</td>
<td>4.25 ± 0.50</td>
<td>70</td>
</tr>
<tr>
<td>GNMG</td>
<td>62</td>
<td>56</td>
<td>06</td>
<td>25</td>
<td>96.0</td>
<td>1.49 ± 0.18</td>
<td>00</td>
</tr>
<tr>
<td>LSD (P&lt;0.01)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.53</td>
<td></td>
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</table>

* Based on 50 explants, ± SD = Standard deviation
levels of phytohormones (Looney et al., 1988; Alvarez et al., 1989; Gronroos et al., 1989).

In the second experiment, BA at 6.66, 8.88 and 11.1 µM resulted into 100, 96.66, and 96.66% of explants showing budbreak. There was marginal difference in the response of BA levels from 0.04 to 0.89 µM. However, a linear increase in number of two or more shoots per explant was observed on increase in BA concentration from 0.04 to 11.1 µM. Maximum response (90%) of two or more shoots per explant was recorded with BA at 11.1 µM (Fig. 1). Addition of BA in MS not only induced bud break in higher number of nodal explants but shoots were of better quality in terms of vigour and leaf colour. Positive influence of BA in establishment of axenic shoots in grapes has earlier been documented in several reports (Chee and Pool, 1983; Reisch, 1986; Lee and Wetzstein, 1990; Robacker and Chang, 1992; Torregrosa and Bouquet, 1995; Mhatre et al., 2000). A second crop of shoots could be induced in mother explants cultured on WPM or MS with BA at 4.44 or 8.88 µM. The maximum shoot induction was obtained in MS with BA (8.88 µM) considering both single and two or more shoots in explants (data not shown).

Primary shoot used as explant, induced maximum multiple shoots (2.27) per explant on an average in MS supplemented with BA (8.88 µM) after 30 days of inoculation (S0). Though marginally higher, a linear increase in number of shoots was observed on increase in BA concentration from 2.22 to 8.88 µM though reverse was true for number of shoots elongated per explant. Medium without BA (served as control) showed the least number of shoots as well as least number of elongated shoots per explant. On transfer of these shoots to fresh media (S1) in glass bottles, number of multiple shoots increased several fold and showed linear increase with increase in BA concentration (Fig. 2A). The same trend was observed with number of elongated shoots per explant (Fig. 2B).

It was observed that BA concentrations at 6.66 and 8.88 µM showed higher number of shoots and elongated shoots per explant from subcultures S0 to S1 however, shoots produced were hyperhydric and showed abnormalities in leaf shape. The leaves were dark green with glossy appearance. Also, shoots
Table 2. Effect of basal media and BA on shoot elongation in cv. Red Globe

<table>
<thead>
<tr>
<th>Basal medium + BA (µM)</th>
<th>Percent of shoots elongated*</th>
<th>Average shoot length (cm) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPM + BA (4.44)</td>
<td>66.67</td>
<td>7.50 ± 0.45</td>
</tr>
<tr>
<td>WPM + BA (8.88)</td>
<td>53.33</td>
<td>6.38 ± 0.45</td>
</tr>
<tr>
<td>WPM</td>
<td>13.33</td>
<td>1.50 ± 0.17</td>
</tr>
<tr>
<td>MS + BA (4.44)</td>
<td>93.33</td>
<td>6.75 ± 0.26</td>
</tr>
<tr>
<td>MS + BA (8.88)</td>
<td>61.67</td>
<td>4.50 ± 0.30</td>
</tr>
<tr>
<td>MS</td>
<td>30.00</td>
<td>1.00 ± 0.06</td>
</tr>
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</table>

LSD (P=0.01) 0.56

* Based on 60 explants, ± SD = Standard deviation

supplemented with or without NAA (0.54 - 1.07 µM) or IAA (0.57 - 1.14 µM) or IBA (0.49 - 0.98 µM) or IPA (0.53 -1.06 µM) however, quality of roots was better on incorporation of NAA at 0.54 - 1.07 µM in the medium. Number of days required for rooting was less for the shoots inoculated in the liquid medium as compared to the solidified medium. In MS half or MS full medium devoid of growth regulators, the quality of roots was poor and shoots lacked vigour. Addition of NAA in the rooting medium induced longer roots with primary and secondary branching. This was reflected in the higher survival of rooted shoots (83%) when treated with NAA (data not shown). In earlier reports on grapevine, it was documented that auxin stimulated root initiation but inhibited subsequent root growth (Galzy, 1969), and that its appropriate concentration was of critical importance. In previous studies, it was observed that effects of auxins on rooting depend on mineral composition of the nutrient media (Novak and Juvova, 1983; Zlenko et al., 1995). Root initiation was not influenced by salt concentration, but root growth was enhanced when salt concentration of rooting media was reduced (Harris and Stevenson, 1979).

Ex vitro auxin pulse treatment of in vitro shoots for 10 min induced direct roots. Shoots given a pulse treatment with auxin mixture of IAA (2.85 µM) and NAA (2.70 µM) showed 80% plant establishment. Pulse treatment of IAA (5.7 µM) or NAA (5.4 µM) alone gave rise 73 and 70% establishment, respectively. A mixture of IAA (5.7 µM) and NAA (5.4 µM) resulted into lower percent (53%) of establishment. Shoots directly transferred to potting mixture without any auxin pulse did not induce roots and could not establish. Plants could be acclimatized by the sachet technique which is simple, effective and does not require any sophisticated set-up. It was found that shoots planted in a mixture of coco-peat + soil + sand (1:1:1) showed a plant survival of 75%.

Thus, present communication describes two routes of micropropagation in grapevine cultivar Red Globe. In one route, whole plants could be developed from single node segments by bud break and direct rooting. To our knowledge no such systematic study on basal media has been reported so far for tissue culture of grapevines. In second route, larger number of plants could be obtained by multiple shoot induction, shoot proliferation and ex vitro rooting by auxin pulse treatment. Within seven months period, about 100 single node segments could give rise to about 5442 in vitro shoots and 4354 established plants compared to conventional vegetative cutting method where each three to five node cutting yields only one plant (Fig. 3). Tissue culture plants produced, have been supplied to National
A one step in vitro cloning procedure for Red Globe grape

Research Center for Grapes (NRCG), for its performance in the field. A simple in vitro propagation procedure described here can complement conventional methods, currently being used in propagation of this important grapevine variety.

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References


