Vitis **42** (4), 199–202 (2003)

Influence of pre-bloom sprays of benzyladenine on *in vitro* recovery of hybrid embryos from crosses of Thompson Seedless and 8 seeded varieties of grape (*Vitis* spp.)

P. V. Bharathy[1)], G. .S. Karibasappa[2)], A. B. Biradar[2)], D. D. Kulkarni[1)], A. U. Solanke[3)], S. G. Patil[3)] and D. C. Agrawal[1)]

[1)] Plant Tissue Culture Division, National Chemical Laboratory, Pune, India
[2)] National Research Centre for Grapes, Manjri, Pune, India
[3)] Agharkar Research Institute (MACS), Pune, India

Summary

The present investigations are part of a study to introgress downy mildew resistance in Thompson Seedless through breeding and *in ovulo* embryo rescue methods. The influence of sprays of benzyladenine (BA) at pre-bloom and bloom on the percentage of embryo recovery, germination and development of hybrid plants is described. BA gave mixed results among the 8 cross combinations. With some crosses, BA drastically improved these parameters. In almost all crosses, germination increased significantly with BA sprays. The influence on plant development and recovery depended on BA treatment and the male parent.

Key words: Benzyladenine, breeding, *in ovulo* embryo rescue, Thompson Seedless, *Vitis*.

Introduction

Downy mildew (*Plasmopara viticola* Berl. et de Toni) is one of the most devastating fungal diseases affecting grapevines worldwide. The control of the fungus involves the repeated application of chemical fungicides and labour-intensive viticultural practices. The use of fungicides, on a large scale, adversely affects the environment. To combine fruit quality of *Euvitis* and the disease tolerance of *Muscadine* grapes intergeneric and interspecific hybridization forms the basis of development of new, disease resistant cultivars of good quality. However, success in terms of useful cultivars is yet to be realized, mainly due to the differences in chromosome number between the two genera and embryo abortion. *In ovulo* embryo rescue thus provides a solution not only for obtaining interspecific crosses, but also allows to use seedless vines as female parents (Cain *et al.* 1983; Emershad and Ramming 1984; Gray *et al.* 1990).

The objective of the present investigations was to study the effect of sprays of BA at pre-bloom and bloom on the percentage of embryo recovery, embryo germination and development of hybrid plants.

Material and Methods

Breeding work was initiated by identification of both male and female parental plants in the germplasm maintained at the National Research Centre for Grapes, Pune. Twenty four vines, cv. Thompson Seedless were selected as female plants. Eight male parental lines (seeded) showing field tolerance to downy mildew were selected as follows: Lake Emerald, Concord, Catawba (belonging to *Vitis labrusca*), Frühroter Veltliner (*Vitis vinifera*), Seyve Villard - S.V. 18402 (*Vitis* spp.), *Vitis tilifolia*, *Vitis candicans* and St. George (*Vitis rupestris* du Lot).

The first spray of BA (30 ppm) was given to parrot green stage panicles, about 10 d prior to emasculation or flowering, followed by the second spray of BA (30 ppm) after 7 d. Emasculation in Thompson Seedless (female) was carried out, followed by immediate bagging of emasculated panicles and subsequent hand cross pollination using a hair brush the next morning with the designated male pollen. This had been collected and stored earlier for each cross combination.

Berries from crosses were collected 40 d after pollination (Figure, A). Berries were washed with liquid soap for 10 min, then were surface sterilized with a mercuric chloride (0.1 % w/v) solution for 10 min and were rinsed 3 times with sterile distilled water. Ovules from the berries were aseptically excised and cultured in petri dishes (15-18 ovules per dish) containing Emershad and Ramming medium (ER) (Figure, B) (Emershad and Ramming 1984), with sucrose (6 %) and activated charcoal (0.3 %). The pH of the medium was adjusted to 5.8 before autoclaving. The total number of ovules inoculated per cross was recorded. The petri dishes were kept under permanent diffused light (6.1 µmol m^{-2} s^{-1}) at 25 ± 2 °C for 10 weeks to allow the development of embryos within the ovule. The experiment was set in a completely randomized design, replicates being either single ovules or embryos.

After 10 weeks, the ovules were dissected aseptically under microscope and embryos were excised from the micropylar end of the ovule. The embryos were transferred to petri dishes containing Woody Plant Medium (WPM)

Correspondence to: Dr. D. C. Agrawal, Plant Tissue Culture Division, National Chemical Laboratory, Pune - 411 008, India. Fax: +91-20-589-3438. E-mail: dagrawal@dalton.ncl.res.in

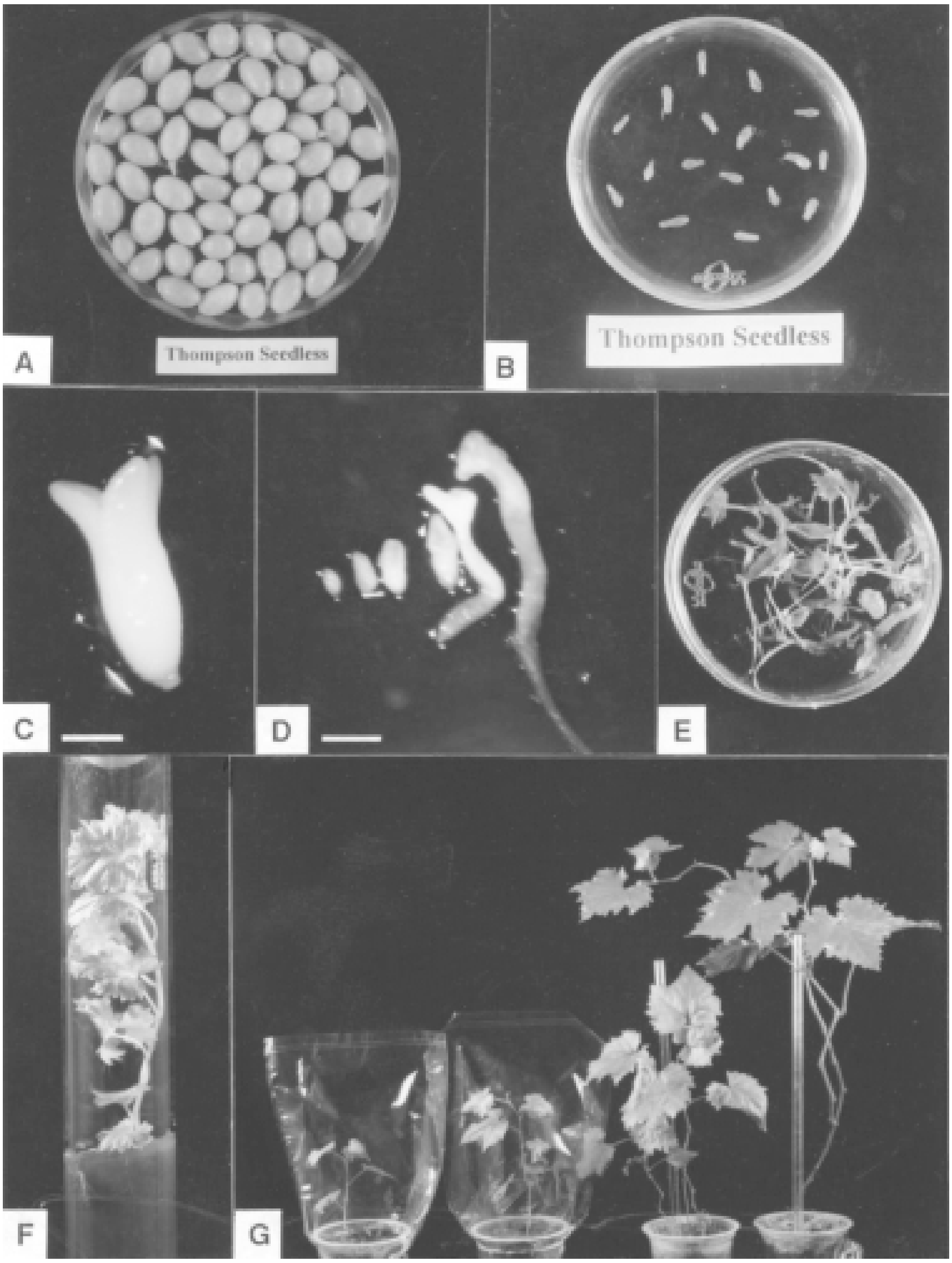

Figure: **A**: Immature berries. **B**: Ovules. **C**: Embryo (bar corresponds to 80 µm). **D**: Stages of embryo development and germination (bar corresponds to 330 µm). **E**: Early stages of plantlets. **F**: Hybrid plant in test tube. **G**: Hybrid plants - stages of hardening.

(LLOYD and MCCOWN 1981), supplemented with BA (1 µM), sucrose (1.5 %), activated charcoal (0.3 %). The medium pH was adjusted to 5.8. Embryos were incubated at 25 ± 2 °C under permanent fluorescent light (12.2 µmol m^{-2} s^{-1}). Germinated embryos, showing the first pair of leaves (Figure, E), were transferred to test tubes (Figure, F) containing WP medium, supplemented with BA (1 µM) and kept under similar light and temperature conditions in order to develop to plants. After 4 weeks, the plantlets were transferred to plastic cups containing a mixture of soil and sand (1:1). Initially these cups were covered with thin and transparent polythene bags (Figure, G) and kept under permanent high light (24.4 µmol m^{-2} s^{-1}) at 25 ± 2 °C for hardening. Survival of the plants was recorded.

Results and Discussion

The results pertaining to the influence of BA on embryo recovery, germination percentage and development of hybrid plants are presented in the Table.

Recovery of embryos: The ovules were mostly hollow and sometimes endosperm was present. Embryo recovery significantly increased by treatment with BA in most of the crosses. Sprays of BA enhanced the percentage of embryo recovery by more than twofold in crosses TS x Lake Emerald, TS x Concord, TS x *V. candicans* and TS x SV 18402 while the reverse was true for the other cross combinations. Maximum embryo recovery (47.57 %) was obtained for TS x Concord followed by TS x SV 18402 (29.75 %). Negative

T a b l e

Influence of benzyladenine (BA) sprays at pre-bloom and bloom on embryo recovery, germination and plant development in crosses between Thompson Seedless and 8 seeded grape cultivars. (S.E. = 7.88, 6.18 and 6.02, C.D. = 23.89, 18.76 and 18.26 at 5 %, for % embryo recovery, germinated embryos and plants developed, respectively)

No.	Cross Thompson Seedless (female) x male	Controls (without benzyladenine treatment) No. of berries excised	No. of ovules cultured	No. of embryos recovered	Response (%) based on ovules: Embryos recovered	Germinated embryos	Plants established in soil	Treatment with benzyladenine No. of berries excised	No. of ovules cultured	No. of embryos recovered	Response (%) based on ovules: Embryos recovered	Germinated embryos	Plants established in soil
1	Lake Emerald (*V. labrusca*)	435	627	24	3.83	1.75	1.12	260	324	55	16.98	16.98	16.98
2	Concord (*V. labrusca*)	138	192	28	14.58	6.25	6.25	162	185	88	47.57	38.92	36.76
3	Catawba (*V. labrusca*)	248	391	54	13.81	11.51	9.46	137	168	20	11.9	11.91	11.91
4	Frühroter Veltliner (*V. vinifera*)	110	158	18	11.39	0.63	0.63	297	395	27	6.84	6.84	6.84
5	St. George (*V. rupestris*)	69	136	18	13.24	11.03	11.03	179	305	21	6.89	6.89	4.92
6	*V. tilifolia*	101	140	9	6.43	5.71	5.71	194	268	1	0.37	0.37	0.37
7	*V. candicans*	29	48	3	6.25	6.25	6.25	269	423	53	12.53	12.29	7.57
8	SV 18402 (*Vitis* spp.)	731	1185	128	10.8	8.95	7.51	251	279	88	29.75	20.10	18.64

effects of BA on embryo recovery was very drastic in cross TS x *V. tilifolia.* The embryos were white and glossy (Figure, C) and situated at the micropylar end of the ovule. All stages of the embryos ranging from globular to cot-leafed were obtained. Pre-anthesis spray of auxins and cytokinins have been reported to enhance seed setting in seedless cultivars of grape (Pandey and Pandey 1990).

E m b r y o g e r m i n a t i o n : The germination percentage of embryos obtained from treated berries was significantly higher than that from untreated ones. In most of the treated crosses, all the embryos germinated except in crosses TS x Concord, TS x *V. candicans* and TS x SV 18402. Only one embryo was recovered from the treated cross TS x *V. tilifolia*, which germinated normally. The embryos germinated within 5 d (Figure, D), although globular embryos took a longer time or did not germinate at all. The root was a taproot in the beginning, but later developed to an adventitious root system. The hypocotyls elongated and turned green, along with the cotyledons. In crosses TS x Concord and TS x Catawba, some of the embryos germinated but became contorted in shape and did not develop epicotyle and leaves in treated lots compared to the untreated ones. Gray *et al.* (1990) also found similar results in grape embryos growing on medium containing BA (1 µM). They found that although the growth pattern of the embryos was abnormal, BA significantly increased embryo germination.

In untreated lots, percentage of germination varied among the crosses (Table). Out of 8 crosses, only TS x *V. candicans* showed 100 % germination. In other crosses, the percentage of germination was poor. The percentage of germination was very poor (0.63 %) in cross TS x Frühroter Veltliner as compared to embryo recovery (11.39 %). The poor germination observed in general in untreated lots may be due to the difference in genetic compatibilities between parental germplasm as reported by Gray *et al*. (1990) and/or dormancy. Grape seeds generally exhibit dormancy which can be broken by cold stratification (Flemion 1937) and the application of exogenous growth regulators like cytokinins (Gray and Mortenson 1987).

D e v e l o p m e n t o f h y b r i d p l a n t s : Plantlets of TS x SV 18402, TS x Concord and TS x Catawba showed vigorous growth as compared to the other crosses, in both treated and untreated lots. Development of plants was significantly higher in BA-treated lots. For the majority of the crosses in both treated and untreated lots, the number of hybrid plants developed commensurated with the recovery of embryos and the percentage of germination, hence it is difficult to infer that sprays of BA had any separate effect in this regard. The differences in plant development among crosses may be associated with the male genotypes since, treated or untreated, SV 18402, Concord and Catawba resulted in a higher plant recovery. The differences between embryo recovery, germination and plant establishment, though interrelated to a certain extent, appear to be independent events and may express varied responses during culture.

From the present investigations, it is evident that sprays at pre-bloom and bloom had a positive effect on embryo development and recovery. Higher rates of embryo recovery, germination and plant development were obtained when

BA was sprayed. The response, however, varied between the cross combination. A positive influence of BA on differentiation and proliferation of dormant "accessory" buds (HAZRA *et al*. 2001), induction of multiple shoots in embryo axes (BANERJEE *et al.* in press) and in cotyledonary nodes (AGRAWAL *et al*. 1997) have been reported earlier for cotton. However, effects of other factors like genetic compatibilities between the parental lines cannot be ruled out. Results obtained may be applied in grapevine breeding programmes, whereby a higher embryo recovery, germination and hybrid plant development may be obtained by application of BA.

Acknowledgement

The financial assistance in the form of research grant by the National Agriculture Technology Programme (NATP), ICAR, New Delhi, India, is gratefully acknowledged.

References

AGRAWAL, D. C.; BANERJEE, A. K.; KOLALA, R. R.; DHAGE, A. B.; KULKARNI, A. V.; NALAWADE, S. M.; HAZRA, S.; KRISHNAMURTHY, K. V.; 1997: *In vitro* induction of multiple shoots and plant regeneration in cotton (*Gossypium hirsutum* L.). Plant Cell Rep. **16**, 647-652.

BANERJEE, A. K.; AGRAWAL, D. C.; NALAWADE, S. M.; HAZRA, S.; KRISHNAMURTHY, K. V.; 2003: Multiple shoot induction and plant regeneration from embryo axes of six cultivars of cotton (*Gossypium hirsutum* L.). Biol. Plant. (in Press).

CAIN, D. W.; EMERSHAD, R. L.; TARAILO, R. E.; 1983: *In ovulo* embryo culture and seedling development of seeded and seedling development of seeded and seedless grapes (*Vitis vinifera* L.). Vitis **22**, 9-14.

EMERSHAD, R. L.; RAMMING, D. W.; 1984: *In ovulo* embryo culture of *Vitis vinifera* L. cv. Thompson Seedless. Am. J. Bot. **71**, 837-877.

FLEMION, F.; 1937: After ripening at 5 °C favours germination of grape seeds. Contr. Boyce Thompson Inst. **9**, 7-15.

GRAY, D. J.; MORTENSEN, J. A.; BENTON, C. M.; DURHAM, R. E.; MOORE, G. A.; 1990: Ovule culture to obtain progeny from hybrid seedless bunch grapes. J. Am. Soc. Hortic. Sci. **115,** 1019-1024.

GRAY, D. J.; MORTENSON, J. A.; 1987: Initiation and maintainence of long term somatic embryogenesis from anthers and ovaries of *Vitis longii* 'Microsperma'. Plant Cell Tiss. Org. Cult. **9**, 73-80.

HAZRA, S.; AGRAWAL, D. C.; BANERJEE, A. K.; KRISHNAMURTHY, K. V.; NALAWADE, S. M.; 2001: Induction of multiple shoots and plant regeneration from accessory buds of nodal segments from field grown mature cotton plants (*Gossypium hirsutum* L.). In Vitro Cell. Dev. Biol. Plant **37**, 830-834.

LLYOD, G.; MCCOWN, B.; 1981: Commercially feasible micropropagation of mountain laurel *Kalmia latifolia*, by use of shoot tip culture. Proc. Int. Plant Prop. Soc. **30**, 421-427.

PANDEY, R. M.; PANDEY, S. N.; 1990: Flowering, fruit set and berry growth. In: R. M. PANDEY, S. N. PANDEY (Eds.): The Grape in India. Indian Council of Agricultural Research, New Delhi.

Received August 4, 2003