

***In vivo* development of ovule in seedless and seeded cultivars of grâpes (*Vitis vinifera* L.) — a particular reference to *in ovulo* embryo culture**

by

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Die Entwicklung der Samenanlagen bei kernlosen und kernhaltigen Rebsorten (*Vitis vinifera* L.) *in vivo* im Hinblick auf die Embryokultur *in vitro*

Zusammenfassung: Um das geeignetste Entwicklungsstadium der Embryonen für ihre *in-vitro*-Kultur zu ermitteln, wurde bei den drei kernlosen Rebsorten Perlette, Thompson Seedless und Beauty Seedless sowie der kernhaltigen Sorte Anab-e-Shahi die Entwicklung der Samenanlagen verfolgt. Die Anzahl geschrumpfter Samenanlagen begann 20 d nach der Anthese anzusteigen. Gleichzeitig setzte bei allen kernlosen Sorten die Abnahme der vitalen Samenanlagen ein. Von den kernlosen Sorten zeigten Thompson Seedless und Beauty Seedless das schwächste Wachstum der Samenanlagen. Bei Anab-e-Shahi entwickelten sich die Samenanlagen schneller als bei den drei kernlosen Sorten. Bei Beauty Seedless und Perlette ging die Gesamtzahl der Samenanlagen 20 bzw. 30 d nach der Anthese zurück. Unter Berücksichtigung der oben genannten Entwicklungszeiten sollten die Embryonen binnen 20 d nach der Anthese in Kultur genommen werden, um aus den später absterbenden Samenanlagen *in-vitro*-Pflanzen zu gewinnen.

Key words: table grape, seedlessness, development, berry, ovule, embryo, tissue culture.

Introduction

Seedless table grapes are preferred by consumers in many markets of the world to an increasing extent. Conventional breeding of seedless grapes involves pollinating seeded grapes with pollen of seedless cultivars. The resulting progenies exhibit 10—15 % seedless progeny (WEINBERGER and HARMON 1964; LOOMIS and WEINBERGER 1979). It has been suggested that intercrossing of seedless grapes to obtain seedless progeny would be more effective. Many seedless grapes are stenospemic (STOUT 1936), in which fertilization occurs but embryo development stops soon after and the seed aborts. Embryo abortion at an early stage seems to be involved in stenospemicity (WINKLER *et al.* 1974; EINSET and PRATT 1975). The degree of embryo development in fertilized ovules of cv. Thompson Seedless ranges from few cells to globular stage (STOUT 1936). The embryo and/or endosperm breakdown in stenospemic grapes occurs about 3 weeks after full bloom. *In ovulo* embryo culture provides an attractive alternative to conventional method of breeding seedless table grapes by allowing recovery of progeny from abortive ovules of seedless × seedless crosses. Further, it would facilitate genetic studies leading to a clearer understanding of the inheritance and nature of the seedless fruit. In the last decade, in various countries, the ovules of seedless grape cultivars have been cultured *in vitro* 14—101 d (CAIN *et al.* 1983), 43—69 d (SPIEGEL-ROY *et al.* 1985), 40—60 d (GRAY *et al.* 1987), 60—70 d (EMERSHAD *et al.* 1989), 52—66 d (TSOLOVA 1990) and 30—60 d (GRAY *et al.* 1990) after anthesis, resulting in rescued growth and/or regenerated plantlets. The age of embryos/ovules at the time of culturing affects the

survival of cultured ovule and its subsequent development *in vitro* (SINGH *et al.* 1991 and BRAR *et al.* 1991).

Culturing of ovules at their early stages of development led to poor survival of the *in vitro* cultured ovules, whereas culturing at the later stages of development is not possible because of embryo abortion or shrivelling of ovules. The paucity of information on the proper developmental stage of embryo at which these should be cultured led to the present investigation. This information is the prerequisite for the development of protocol of *in ovulo* embryo culture of seedless grapes in North-Western India.

Materials and methods

The present studies were undertaken in the Department of Horticulture, Punjab Agricultural University, Ludhiana, India (30° 56' N, 75° 52' E) in Northern India. 8 years old uniform grapevines of seedless cvs Perlette, Thompson Seedless, Beauty Seedless and seeded cultivar Anab-e-Shahi were selected. The vines of all the cultivars were given similar cultural practices including fertilization, irrigation and plant protection measures (ANONYMOUS 1988). Inflorescences were tagged at full bloom stage in all the cultivars. The berries from the open pollinated panicles were collected up to 45 d post anthesis at 10 or 5 d intervals. The rate of berry development (diameter of berry) was recorded (10—45 d post anthesis). The ovules were excised from the seeded and seedless cultivars at various developmental stages. The number of ovules per berry, shrivelled and viable ovules per berry and length of the ovules were recorded. The averages of these parameters were worked out along with their standard deviations.

Results and discussion

Development of berry

The linear increase in the diameter of berries was recorded up to 30, 35 and 45 d post anthesis in Thompson Seedless and Anab-e-Shahi, Perlette, and Beauty Seedless, respectively. Later on, slow growth phase of berry and the following linear increase in berry diameter were variable in these cultivars (Fig. 1). Similar results have also been reported on the comparative development of seeded and seedless cultivars of grapes by BHULLAR (1973). SPIEGEL-ROY *et al.* (1985) also reported the linear increased berry weight till 46 d post anthesis in different cultivars of grapes.

Viable and shrivelled ovules

The number of viable ovules per berry started declining 20 d post anthesis in all the three seedless cultivars (Fig. 1). The rate of decrease in number of viable ovules per berry was abrupt in cultivar Beauty Seedless and Thompson Seedless between 20 and 30 d post anthesis. The rate of decrease in number of viable ovules per berry was slow in cv. Perlette as compared to Beauty Seedless and Thompson Seedless. The viable ovules started declining 35 d post anthesis in cv. Anab-e-Shahi. The shrivelling of ovules started 20 d post anthesis even in Anab-e-Shahi, a seeded cultivar, but it was negligible till 35 d post anthesis. It was increased to a single shrivelled ovule per berry 40—45 d post anthesis. As in the seeded cultivar, the shrivelling of ovules started 20 d post anthesis in all three seedless cultivars.

The abrupt increase in the number of shrivelled ovules was recorded onward 20 d post anthesis in Beauty Seedless, Thompson Seedless and Perlette (Fig. 1). Likewise, NITSCH *et al.* (1960) and BARRITT (1970) reported that embryo and/or endosperm breakdown in stenospermic grapes occurred about 3 weeks post full bloom. The inverse relationship has been exhibited between shrivelled and viable ovules in seedless cultivars, whereas such an inverse relationship was lacking in cv. Anab-e-Shahi in spite of the presence of viable and shrivelled ovules in the berry.

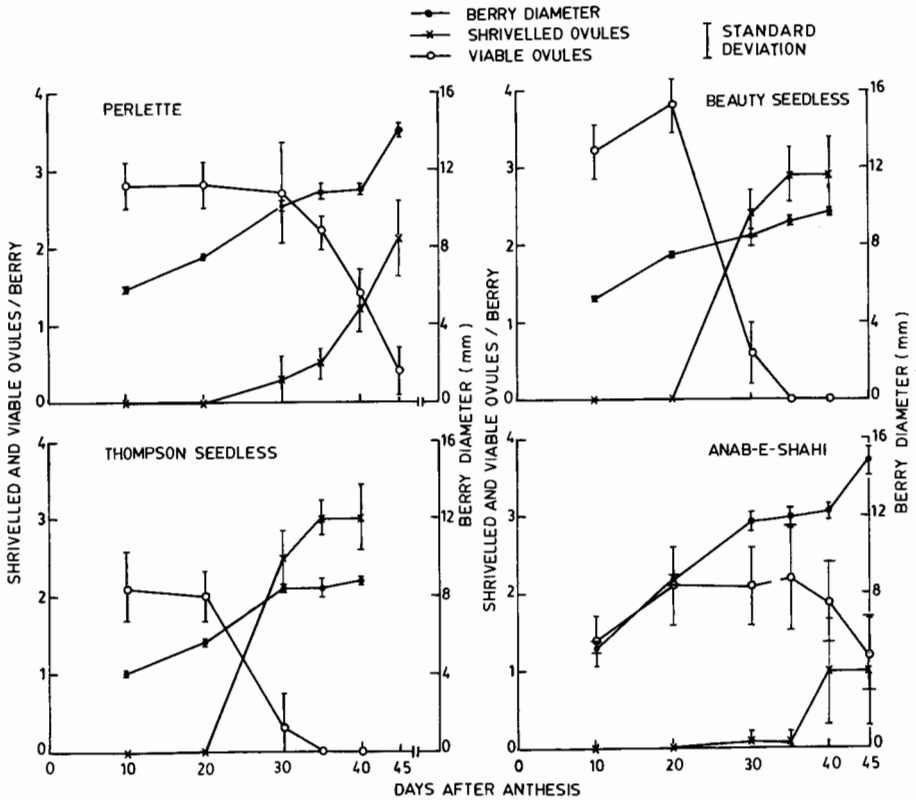


Fig. 1: Berry diameter and number of shrivelled and viable ovules per berry in seedless and seeded cultivars of grapes (*Vitis vinifera* L.) 10–45 d after anthesis.

Beerendurchmesser sowie Anzahl geschrumpfter und vitaler Samenanlagen je Beere bei kernlosen und kernhaltigen Rebsorten (*Vitis vinifera* L.) 10–45 d nach der Anthese.

Development of shrivelled and viable ovules

The rate of increase in the length of ovules of Anab-e-Shahi was highest as compared to all the three seedless cultivars (Fig. 2). Amongst seedless cultivars, the rate of increase in the length of ovule was lowest in Beauty Seedless and Thompson Seedless as compared to Perlette. The restricted growth of the ovule in these seedless cultivars is possibly due to degeneration of the embryos in ovules which subsequently affect various physiological processes and eventually reduced growth of developing ovules. Earlier, STOUT (1936) reported the degree of embryo development in fertilized ovule ranging from few cells stage to globular stage of the embryo in cv. Thompson Seedless.

Later on, NITSCH *et al.* (1960) and BARRITT (1970) also reported that embryo and/or endosperm breakdown occurred 3—4 weeks after full bloom in seedless grapes.

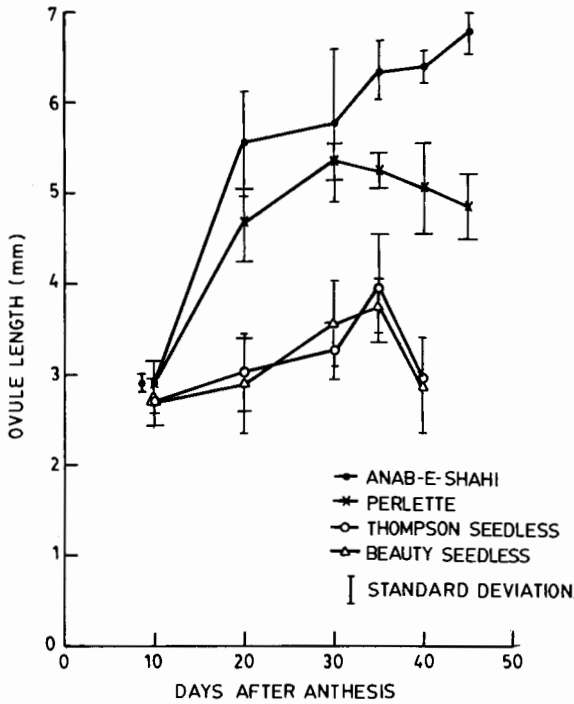


Fig. 2: Development of ovules in seeded and seedless cultivars of *Vitis vinifera* L. (10—45 d after anthesis).

Entwicklung der Samenanlagen bei kernhaltigen und kernlosen Sorten von *Vitis vinifera* L. (10—45 d nach der Anthese).

Number of ovules per berry

Total number of developing ovules per berry declined 30 and 20 d post anthesis in cv. Perlette and Beauty Seedless (Fig. 3), whereas in cv. Anab-e-Shahi the number of developing ovules per berry did not decline 20 d post anthesis. The decline in the total number of developing ovules in seedless cultivars may be attributed to the degeneration of the embryo, eventually the process led to ovules collapsing and/or seed abortion. This phenomenon may be responsible for the reduction in number of developing ovules per berry in Perlette and Beauty Seedless. The collapsing of developing ovules has also been reported in seedless grapes by BARRITT (1970). Unlike to Perlette and Beauty Seedless, the total number of developing ovules per berry did not decline in Thompson Seedless even 30 d post anthesis. However, in this cultivar an abrupt decrease in the number of viable ovules per berry and a substantially increased number of shrivelled ovules per berry has been observed 20 d post anthesis (Fig. 1).

Possibly, in many of ovules of Thompson Seedless, the embryo failed to develop completely but did not degenerate before the fruit was mature. Similar events of ovule development in seedless grapes have also been reported by BARRITT (1970). Total number of developing ovules per berry did not decline much in Anab-e-Shahi.

Keeping in view the number of shrivelled ovules per berry, viable ovules per berry and growth of viable ovules at various developmental stages of berry, it is concluded that the embryo may be rescued *in vitro* prior to 20 d post anthesis in cvs Perlette, Thompson Seedless and Beauty Seedless. *In ovulo* embryo rescue at the developmental stage may allow the recovery of progeny from seedless \times seedless crosses of grapes. Proper inheritance studies can be accomplished by heretofore impossible direct combination of seedless cultivars of grapes.

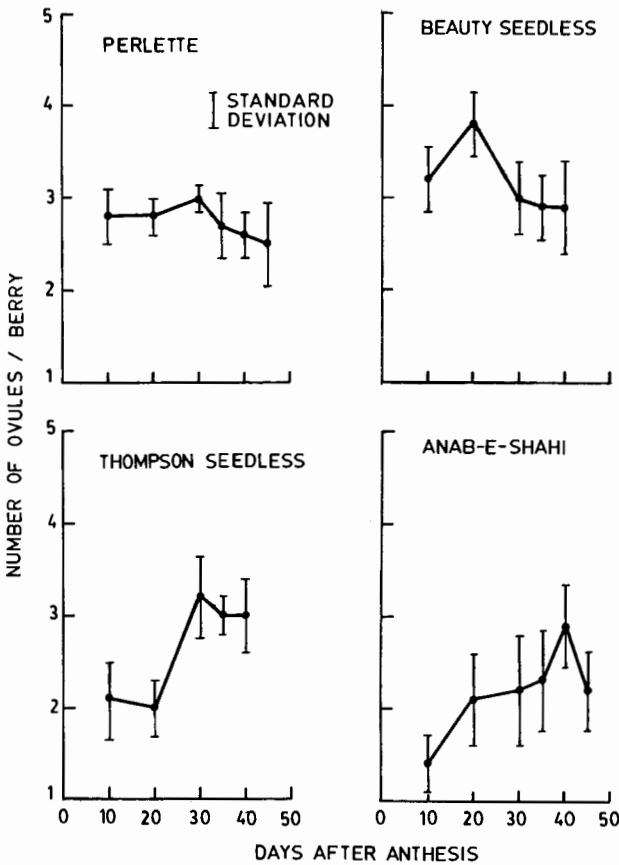


Fig. 3: Number of ovules per berry at various developmental stages (10—45 d after anthesis) in seedless and seeded cultivars of *Vitis vinifera* L.

Anzahl der Samenanlagen je Beere in verschiedenen Entwicklungsstadien (10—45 d nach der Anthese) bei kernlosen und kernhaltigen Sorten von *Vitis vinifera* L.

Summary

Ovule development was examined in the three seedless cvs Perlette, Thompson Seedless, Beauty Seedless and the seeded cv. Anab-e-Shahi, to identify the proper developmental stage of the embryo for *in ovulo* embryo culture. The number of shriv-

elled ovules started increasing 20 d post anthesis. At the same time, the number of viable ovules started declining in all the seedless cultivars. Amongst seedless cultivars, the growth of ovules was least in cvs Thompson Seedless and Beauty Seedless. The ovule development was faster in Anab-e-Shahi as compared to all the seedless cultivars. Total number of ovules per berry declined 20 and 30 d post anthesis in cvs Beauty Seedless and Perlette, respectively. Keeping in view the above mentioned parameters, the embryos in seedless cultivars of grapes may be rescued *in vitro* prior to 20 d post anthesis to obtain plantlets from these abortive ovules.

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