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RECEIVING SEEDLESS VARIETIES OF GRAPES BY IN VITRO METHOD

Abstract

The results of the study of embryo culture by crossing seedless varieties and promising hybrid forms of Turkish grape selection.

Key words: seedless grapes, variety, hybrid plantlets, embryo culture, interbreeding, nutrient medium.

Introduction

Stenospermic grapevines, from which seedless but otherwise normal fruit develop after fertilization, form the germplasm base for breeding new seedless cultivars. Using standard breeding techniques, seedless cultivars can only be used as pollen parents. However, with ovule culture it is now possible to use seedless vines as females. This approach dramatically increases potential germplasm combinations and allows heretofore impossible crosses between seedless cultivars. Two methods of embryo culture have been developed. One used liquid culture in which ovules are placed on filter paper supports [1]; the other employed solidified medium [2]. Each method has been successfully used for *Vitis vinifera.* However, there are various regionally adapted seedless cultivars that are hybrids between *V. vinifera* and other native *Vitis* species [3]. that could be used as maternal parents with the aid of ovule culture. One such hybrid is 'Orlando Seedless', which is the only seedless cultivar resistant to Pierce's disease and adapted to tropical and subtropical climates [4]. Use of 'Orlando Seedless' in seedless x seedless crosses should lead to more rapid development of improved seedless cultivars adapted to the southeastern United States and Caribbean region. A previous study found solid medium culture of 'Orlando Seedless' preferable to culture on liquid medium [5]. In this report, refinements of the system and development of embryos into plants are described, with emphasis on 'Orlando Seedless' and other seedless hybrid cultivars. Isozyme data to confirm parentage, as well as preliminary notes on field performance of the resulting progeny, are included.

The purpose of research to get seedless table grape varieties.

Material and methods

Plant materials and preparation of ovules. Seedless berries of *V. vinifera* grapes 'Alfons', 'Uslu', '1C/2' and '2B/56' were harvested about 6 weeks after anthesis. Immature berries of muscadine grape cultivars were harvested from the experimental vineyard at the department of horticulture, Akdeniz University, Antalya, Turkey. Berries were surface sterilized by immersion in the solution: 60% a cleaning agent Domestos and 40% distilled water with a few drops of Tween 20 for 20 min, and washed three times with sterilized distilled water for 15 min. After sterilization, ovules were extracted from berries and scratched slightly on the surface under sterile conditions then placed onto three different media in 15 × 100 mm Petri dishes. Twenty ovules were placed on each petri dish, and 10 Petri dishes were used per treatment.

In vitro culture conditions. The media E20A used were: 1) macroelements: KNO_3, NH_4NO_3, $MgSO_4x7H_2O$, $CaCl_2x2H_2O$, KH_2PO_4, $Ca(NO_3)_2x4H_2O$, $NaH_2PO_4x4H_2O$, $(NH_4)_2SO_4$, KCl; 2) microelements: $MnSO_4x7H_2O$, $ZnSO_4x7H_2O$, H_3BO_3, KI, $NaMgO_4x2H_2O$, $CuSO_4x5H_2O$, $CoCl_2x6H_2O$, $Na_2EDTAx2H_2O$, $FeSO_4x7H_2O$; 3) organic substances: Myo-Inositol, Pyrodoxine-HCl, Nicotinic acid, Thiamine-HCl, Calcium Pantothenate, Biotine, Glycin. pH was adjusted to 5.9 prior to addition of agar and sterilized at 121°C and 104 kPa pressure for 20 min. Cultures were maintained at 25 ± 2°C under cool-white fluorescent lamps providing with 16 h photoperiod. Cultures were evaluated after four weeks of culture, and observed on a weekly basis.

Results and discussion

Mean length of ovules from pollinated berries of '2B/56' increased from 0.75 mm at 10 days after pollination to 2.2 and 4.8 mm at 20 and 40 days, respectively. This increase indicates continuous growth and development of ovules during berry maturation and confirms similar observations for seedless V. vinifera where growth was measured by weight increase. The latest developmental stage at which ovules could be conveniently dissected was immediately before berry softening. Cultured ovules possessed a papery inner integument surrounding endosperm or embryos. A multilayered outer integument with both a fleshy outer layer and a slightly sclerified inner layer composed the ovule wall. This interpretation is consistent with descriptions of integument development for seeded grapes. Abortive ovules appear to be similar to nonabortive ovules with respect to occurrence of multilayered integuments.

In the table 1 shows the characteristics of the grapes used in hybrid breeding. Since our goal is to get seedless varieties, we use similar parental forms (table 1).

Table 1 - Characteristics of hybrids

Name of hybrid	Date of maturation	Weight berries, g	Seed weight, mg	Color of berries	Form of berries	Availability of seeds
Alfons	21-31.07	5.0	32	black	round	seedless
Uslu	12-18.07	3.7	6	white	oval	seedless
1C/2	10-16.08	2.4	1.3	black	round	seedless
2B/56	21-24.08	3.3	9.2	red	oval	seedless

Seed size grapes 'Uslu' impact on the amount received embryo. This can be visually marked on figure 1. For growing embryo was chosen culture medium E20A. It is one of the suitable media for cultivation of seedless grapes.

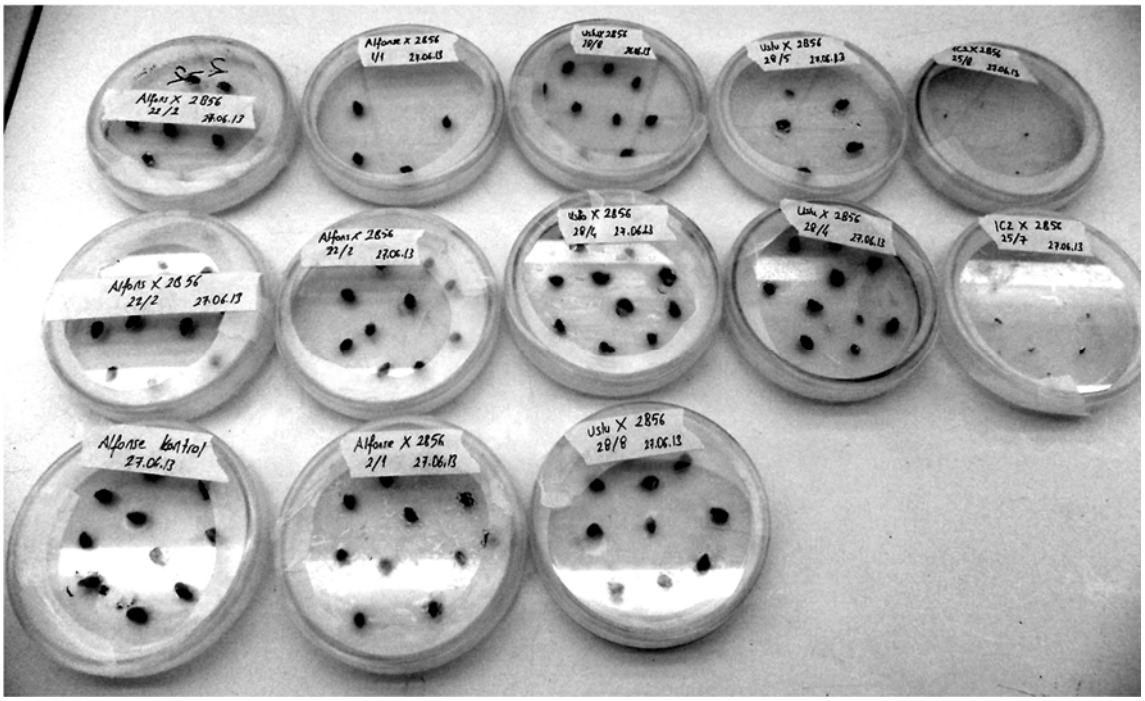

Figure 1 - Embryo inoculated into the culture medium E20A

All stages of embryo development, from globular to torpedo, were recovered. Currently, we allow berries to develop for up to 60 days, but dissect and culture ovules as early as 40 days if berry softening has commenced. This approach allows the greatest degree of ovular development but also considers ease of dissection. Comparison of embryo recovery from varieties and hybrids with '2B/56' pollen parent showed '1C/2' x '2B/56' produced fewer embryos than other crosses when

cultured 42 days after pollination. Both ‘Alfons’ x ‘2B/56’ and ‘Uslu’ x ‘2B/56’ produced the most embryos at 42 days (fig. 1). Reasons for low embryo recovery from ‘1C/2’ x ‘2B/56’ at 42 days are unknown. There were no statistical differences in embryo recovery at the 42-day culture date between reciprocal crosses and ‘1C/2’ and ‘2B/56‘. It is reasonable to assume that different combining abilities among cultivars would lead to differences in embryo recovery.

Conclusions

From the present investigations, it was inferred that ‘Alfons’ was more efficient as the female parent and the number of embryos recovered was nearly double as that of ‘1C/2’. ‘Uslu’ and ‘2B/56’ seem to have a better compatibility with ‘Alfons’ and ‘2B/56’, hence resulting in a higher recovery of embryos, percentage germination and development of hybrid plants.

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ПОЛУЧЕНИЕ БЕССЕМЯННЫХ СОРТОВ ВИНОГРАДА МЕТОДОМ IN VITRO

Резюме Представлены результаты исследования эмбриокультур полученные от скрещивания бессемянных сортов и перспективных гибридных форм винограда Турецкой селекции.

Ключевые слова: бессемянный виноград, сорт, гибридные ростки, эмбриокультура, скрещивание, питательная среда.

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ЖҮЗІМНІҢ ҰРЫҚСЫЗ СҰРЫПТАРЫН IN VITRO ӘДІСІМЕН АЙЫРЫП АЛУ

Түйін Түріктің селекциясының жүзімінің ұрықсыз сұрыпы және болашағы зор буданды пішіндерін будандастырудан алынған эмбриокультураның зерттеу нәтижелері көрсетілді.

Кілт сөздер: ұрықсыз жүзім, сұрып, буданды сабақтар, эмбриокультура, будандастыру, қоректік орта.