Causes of Difference in Success in Reciprocal Crosses between *Vitis vinifera* Linn. and *Vitis rotundifolia* Michx.

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Received August 6, 1956

Introduction

*Vitis vinifera* Linn., *V. labrusca* Linn., and *V. rotundifolia* Michx. are the cultivated species of grape. According to the taxonomic classification presented by Small (1913), Bailey (1934) and Gray (1950), the former two species belong to the subgenus *Euvitis* and the latter to *Muscadinia*. The taxonomists agree that the two—*V. vinifera* L. and *V. rotundifolia* Michx.—cultivated grape species, are very distantly related.

Interspecific hybridization programme was undertaken on a large scale by Patel and Olmo (1955) with a view to transfer the desirable genes—high resistance to diseases and insect pests, and ability to produce a good crop under highly fluctuating climatic conditions—of *rotundifolia* to *vinifera*. It was reported by them that when *vinifera* is used as female and *rotundifolia* as male parent, the crosses are completely successful, while reciprocally it is a total failure.

Therefore, the present investigation was undertaken to determine the causes of this incompatibility.

Materials and methods

The male-sterile *rotundifolia* variety—James—was pollinated with fresh pollen of functional hermaphrodite *vinifera* varieties—Flame Tokay and Malaga. The male-sterile *vinifera* variety—Hunisa—was pollinated with *rotundifolia* I (vine bearing male flowers only). Controlled pollinations were also made in both the species—pollinating Hunisa with Flame Tokay and Malaga in case of *vinifera*, and pollinating James with *rotundifolia* I in case of *rotundifolia*.

Pollen grains of *rotundifolia* I are rather more uniform in size than those of Malaga and Flame Tokay. The germination test of the pollen was made in 20 per cent sucrose solution by hanging drop method before undertaking crossing and it was found to be 15.6, 7.2 and 8.8 per cent in case of Malaga, Flame Tokay, and *rotundifolia* I respectively.

From each type of cross, about twenty-five pistils were collected in glass
tubes at different intervals of time—6, 12, 24, 36, 48, 72 and 96 hours after pollinations and frozen (at 12°C) for later use in studying the pollen tube growth by dissecting the pistils and following different methods of staining. About twenty-five pistils were also fixed in formalin-acetic-alcohol, Carnoy’s solution and Crafoord’s solution for studying the pollen-tube growth and its behaviour and embryo sac formation. After twenty-four hours fixation, the pistils were dehydrated in a butyl alcohol series, infiltrated, and embedded in paraffin.

The frozen pistils were dissected and inspite of having followed different staining techniques, pollen tubes could not be seen. Ten of the embedded pistils from each collection were sectioned at twelve microns. Various staining procedures—as recommended by Nebel (1931) in case of fleshy styles of grapes, cherries and plum, by Raptopoulos (1941) in cherries and by Terami (1930) in pears—for staining pollen tubes and the method suggested by Foster (1934) for cell walls in the meristematic tissues were tried but it remained almost impossible to distinguish pollen tubes from the rest of the stylar tissue. Finally tannic acid, iron chloride and resorcin blue were used in the same series. The slides were stained in 0.25 per cent resorcin blue in 30 per cent alcohol for six hours, and then the same series was followed as given by Foster. The results were encouraging and therefore most of the slides of *vinifera* as well as *rotundifolia* pistils were stained by this procedure. Only the pollen tubes and the germinating pollen grains on the stigma were stained brilliant blue, while the rest of the stylar tissue and the non-germinated pollen grains stained reddish brown.

This work was done at the Agricultural Experimental Station, University of California, Davis, U.S.A.

**Observations**

In all cases the pollen grains start germinating in about six hours after pollination and enter into the stylar tissue. The pollen tubes practically reach the base of the style in about twenty-four hours.

A large number of pollen grains of Malaga and Flame Tokay germinate normally on the stigma of James, while a relatively smaller number of *rotundifolia* pollen grains germinate on the stigma of Hunisa. No perceptible difference in the rate of pollen tube growth and no abnormal features such as bursting of pollen tubes or their branched and swollen condition are observed in any type of the cross. The normal pollen tubes of Malaga and Flame Tokay can be seen clearly in the styles of James twenty-four hours after pollination (figs. 1 and 2). The pollen tubes penetrate the entire style and in a few cases reach the ovules of James pistils after thirty-six hours of pollination.

The style of *rotundifolia* is much longer (600 μ) and rather uniformly thick from stigma to the ovary than that of *vinifera* (400 μ) (figs. 3 and 4).
In case of *vinifera*, style is not uniform in thickness and gradually becoming smaller from the ovary towards the stigma.

Embryo-sac formation is normal in both the species. The polar fusion nucleus moves toward the egg just prior to fertilization. Fertilization is not observed with certainty. In spite of failure to observe fertilization in the sectioned pistils of Hunisa, fertilization definitely takes place in about thirty-six hours after pollination as can be judged from the swelling of the pistils two days after pollination, and also from the numerous seeds produced. Most probably fertilization does not occur in James, because no enlargement of the ovaries was observed even four days after pollination. The pollinated as well as non-pollinated pistils of James are of the same size and shed at about the same time.
Discussion

Total failure to cross *rotundifolia* female with *vinifera* male is not merely due to its longer style length because *vinifera* pollen tubes penetrate the entire style and even in some cases the ovules of *rotundifolia* pistils in the same duration of time as control. However, a few cases are reported in the literature where style length is a factor prohibiting successful hybridization. Focke (1881) reported that *Mirabilis jalapa* (2n=58) can be crossed with *M. longifolia* (2n=58) if the former is used as the female parent but not if it is used as the male. Strasburger (1886) and Jost (1907) further reported that though the pollen grains of each germinate equally well on the stigmas of the other, the tubes of *jalapa* are unable to make their way completely through the long styles of *longifolia* whereas the tubes of *longifolia* easily traverse the short styles of *jalapa*. The same cause has been suggested by Ostenfold (1929) for his repeated failures in crossing *Polynamium pauciflorum* ♀ (2n=18) with *P. mexicanum* ♂ (2n=18), but reciprocal crosses were successful. The style length of *pauciflorum* is about eight times as long as that of *mexicanum*. He further stated that the pollen of *mexicanum* with short style length does not have sufficient growing force to penetrate through the long style of *pauciflorum*. However, there are other cases reported in the literature where failure to cross in one direction is, in spite of having the same style length in the species concerned, due to some other cause. Thompson (1930, 1940) summarises many such reciprocal crosses.

Many investigators have reported that greater success occurs in interspecific crosses if the species with the larger chromosome number is used as female parent. No *rotundifolia* female tried would cross with any *vinifera* male, in spite of having a higher chromosome number in *rotundifolia* (2n=40) than in *vinifera* (2n=38). Thus in this case when the species with the lower chromosome number is used as the female parent, the crosses are completely successful, while reciprocally it is a total failure contrary to the general belief. The other interesting results in this connection are (I) a larger number of *vinifera* pollen grains germinates on *rotundifolia* stigmas, (2) the rate of *vinifera* pollen tube growth in *rotundifolia* style appears the same as that of *rotundifolia* pollen in *vinifera* style, (3) the style length of *rotundifolia* is one and one-half times longer than that of *vinifera* and still all *vinifera* pollen tubes penetrate the entire style and in some cases even reach the ovule, (4) though no fertilization was observed in the sectioned material, it can be surmised from the course of ovary development that it does not occur, because no differences between the pollinated and the non-pollinated pistils were observed and secondly both the pollinated and the unpollinated ovaries shed at the same time. This failure to cross would probably be due to the secretion of some inhibiting substance or substances in the cytoplasm of the embryo-sac or in maternal tissue closely adjacent. Such substance or substances stop the growth of the pollen tube and its
normal discharge when it reaches close to the embryo-sac. Thus the cause of failure to cross could be assigned to the differences in the cytoplasm of the two species. No such abnormal case of incompatibility in interspecific hybridization has been reported.

From this and the previous investigation made by Patel and Olmo (1955), it may be mentioned here that in the course of evolution *rotundifolia* has been isolated from *vinifera* and other species of *Vitis* by various mechanisms such as (I) the difference in the time of blossoming—*rotundifolia* blooms three to four weeks later than *vinifera* and only rarely overlaps with any other species of *Vitis*, (2) the difference in chromosome number—*rotundifolia* has $2n=40$ while other species of *Vitis* have $2n=38$, (3) failure to cross when it is used as a female parent—most species of *Vitis* have been reported to cross with ease in both the directions, (4) difference in style length—the significance of this feature is not fully explored, (5) almost complete sterility of the hybrids—the hybrids reported between various other species of *Vitis* are highly fertile, (6) $F_2$ and $F_3$ hybrids are weak growers, (7) morphological characters are very distinct, as well as physiological ones, since no other species of *Vitis* can be successfully grafted with it.

**Summary**

Styles of *V. rotundifolia* are uniformly thick from stigma to the ovary and one and one-half times longer than that of *V. vinifera*. The differences in the length of styles has nothing to do in the failure of crossing *rotundifolia* female ($2n=40$) with *vinifera* male ($2n=38$), since the pollen tubes of *vinifera* penetrate the entire style and in some cases the ovules of *rotundifolia* pistils in the duration of time as control.

The absence of swelling of the ovaries even four days after cross-pollination with *vinifera* and secondly the abscission of pollinated as well as non-pollinated pistils at about the same time suggests that fertilization does not occur.

The complete failure of crossing, therefore, must reside in the cytoplasm of either embryo-sac or and maternal tissue surrounding the embryo-sac.

**Literature cited**


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