

Full Length Research Paper

Occurrence of star flowers in Cardinal (*Vitis vinifera* L.) CV.

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Star flowers are flowers that differ from normal *Vitis* flowers in that the calyptra breaks open from the top like the flowers of most other species plants. This is the first report of the occurrence of star flowers in Turkey. Star flowers were observed in Cardinal (*Vitis vinifera* L.). The structural features are compared to normal flowers. A genetic analysis using RAPD-PCR was performed to determine if there was a genetic difference between the vines nearing two flower types. There were some minor morphological differences, but RAPD analysis did not show any genetic dissimilarities. Inflorescences appeared more compact on star flowered vines due to shorter pedicels in flowers. Fruit set was also extremely low. This formation is reported in different regions of the world on different cultivars. This might be more common than previously thought. It warrants further research.

Key words: Grape, star flower, genetic analysis, RAPD, cardinal.

INTRODUCTION

A perfect grape flower is the flower with all parts (calyx, corolla, stamens and pistil) present. Perianth—the floral envelope is usually divisible into an outer whorl (calyx) of sepals and an inner whorl of five petals (corolla), also known as calyptra. These petals are fused at the tip; hence the vine flower does not open from the tip, as is the rule with most flowers (Winkler et al., 1974; Ağaoğlu, 1999).

During flowering, some abnormalities in the way of opening of vine flowers have been encountered. This abnormality, casually named as ‘star flower formation’ differs from normal *Vitis* flowers because the calyptra opens from the top in star formation. Star flowers more closely resemble flowers of the genus *Cissus* (Longbottom et al., 2004). Star flowers have been associated with male sterility and poor fruit set (Portele, 1883; Despeissis, 1921; Kozma, 1960; Ağaoğlu, 1969; Pratt, 1971).

Quieiroz-Voltan et al. (1998) reported a structural abnormality in the flowers of Italia (*V. vinifera* L.) cultivar, including the opening of the flowers from the top. Reports about star flower formation in Australia came from

Longbottom et al. (2004, 2008) in the cultivars *V. vinifera* x *Vitis labrusca* cv. Canada Muscat, *V. vinifera* cvs. Gamay, Pinot Meunier, Chardonnay, and Shiraz. In addition, star flower variants were observed by Longbottom et al. (2004) on own-rooted Merlot (clone D3V14) and Cabernet Sauvignon (clone G9V3) vines.

This paper describes the morphological studies on normal and star flower variant Cardinal (*V. vinifera* L.) vines and genetic analysis using RAPD-PCR to determine possible morphological and genetic differences.

MATERIALS AND METHODS

Cardinal vines with star flowers described in this paper were grown in the Experimental vineyard at the Çanakkale Onsekiz Mart University's Yahya Çavuş Campus at Çanakkale, Turkey and were discovered by a casual observation during the vegetative season of 2006. The 0.45 ha vineyard was founded as a collection parcel in 1997. The normal and star flowered Cardinal vines were grafted onto 41B rootstock and trained to bilateral cordon system.

Morphological studies on flower inflorescences and clusters of both normal and star flowered vines were carried out according to the IPGRI, UPOV, OIV (1997) for two successive years. There were only two star flowered vines in the vineyard carrying two-three inflorescences and therefore clusters in total. Five normal flowered vines were also observed for the morphological study. Genetic analysis was performed in the Agricultural Biotechnology Laboratory at Department of Horticulture, Ankara University. As a source

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Table 1. Names and base sequences (5'–3') of RAPD primers used in the detection of polymorphism in normal and star flowered Cardinal grapevines (*Vitis vinifera* L.).

K 1	GCGACCATGG	RAPD 4	GTCCTACTCG	B 389	CGCCCCGAGT
K 2	CAGGCCATGG	RAPD 5	CTACTACTCG	P 33	GTAAAACGACGGCCAGT
K 3	GCCATGGACG	RAPD 6	TCCTCACTAG	P 123	GGGATTTCGAC
K 4	GATGGTACCG	RAPD 7	GTGCTTAGCG	P 313	AAAGCCGTCC
K 5	CGCAGGATGG	RAPD 8	CAGGCCCTTC	P 437	CGGATCGACA
K 6	CGATGACTGG'	RAPD 9	CTACACAGGC	S 34	GATAGCCGAC
K 7	GGGATGGCTG	UBC 237	CGACCAGAGC	S 39	TCGGCCTGCT
K 8	CCCATGGGTG	UBC 238	CTCTCCAGCA	SC 1023	GGCTCGTACC
RAPD 2	GTCCTCAACG	BC 374	GGTCAACCCT	SC 1048	CTGGTATGCG
RAPD 3	CTGATCGTAC	B 379	GGGCTAGGGT	SC 1076	CGCAGACTTG

for DNA, one year old cuttings of both normal and star flower variants were brought to the department's greenhouse and planted into perlite and turf medium. Unopened and young leaves were used to extract DNA according to the method described by Lefort et al. (1998).

Amplification and RAPD reactions were done according to Ergül (2002) in a 25 µl reaction mixture containing 100-200 ng template DNA, 1.5 u Taq DNA polymerase (Promega, Wis.), 0.25 mM of each of four dNTPs, 0.2 µM oligonucleotide primers 10-17 bases long, 500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25°C) and 1% Triton® X-100. The reaction mixture was overlaid with a drop of mineral oil (Sigma, M-5904). Amplification was performed on a thermocycler (PTC-100; MJ Research Inc., Waltham, Mass.) for 35 cycles of 94°C for 30 s, 35°C for 60 s and 72°C for 105 s followed by a final hold of 8 min at 72°C. The amplification products were resolved on 1% agarose plus 1% Nusieve™ (FMC Corp., Maine) agarose gels. Electrophoresis was carried out in 1X TBE (Tris-Boric acid-EDTA) buffer (Sambrook et al., 1989) at 7 V/cm. The RAPD bands were visualized with 0.01 mg ml⁻¹ ethidium bromide under uv light (λ=302 nm) and recorded with Type 65 Polaroid film.

A total of 30 primers were tested to screen DNAs (Table 1). The primers synthesized at Research Genetics (Huntsville, Ala.) were UBC series (237, 238), BC-374, B-379, B-389, P-33, P-123, P-313, P-437, Kozak primers (1-8), S-34, and S-39. Primers obtained from IDT (Integrated DNA Technologies, Inc., Coralville, Iowa) were RAPD series (2-9), and SC series (1023, 1048, 1076). These were chosen because of their high polymorphism detection in the work of Gökbayrak et al. (2006).

RESULTS

Vegetative development

The vegetative growth and development of the normal and star flowered Cardinal vines was similar. However, not noticeable at the first glance, pigmentation in the shoot tips was more intense in the star flowers. Leaf contour of star flowered vines was rough and undulating with wider and longer teeth with rounded sides. Petiolar sinus of the star flowered vines was wider compared with the normal Cardinal vines. Leaf blade and petiole were also shorter in the star flowered vines.

Budbreak on star flowered Cardinal vines occurred one or two days later in both season compared to the normal flowered vines.

Reproductive development

Fourteen and ten inflorescences were born on the star flowered vines in the first and second years of observation, respectively. Number of inflorescence on a shoot was similar to the normal vines (0.97 vs 0.84). However, star inflorescences had more flowers developed on them (172 vs 154). First cluster on the shoot was approximately three centimeters shorter. Pedicels on star flowers were shorter and flowers were bigger giving the cluster a more compact appearance. Statistical analysis could not performed because of the insufficient number of observations on star flowered vines and/or clusters.

Observations made in the vegetative season revealed that some star flowers in an inflorescence had fewer stamens compared to the normal flowers. Filaments on some star flowers were at the same height with the pistil. Anthers occasionally had a pink spot in the middle. Star flowers on Cardinal began opening one or two days later than normal vines. Calyptra on star flowers had pink-red pigmentation at the tips of their petals as the flowering progressed (Figure 1).

Not all inflorescences on star flowered vines had star flowers. A few flowers opening in the normal way were also observed (Figure 2). It was observed that some flowers broke opened at the sides of the calyptra. Normal flowered Cardinal vines provided fruit set as expected in both years. Fruit set was very poor in star flowered vines and all the berries were seedless. Only one cluster had six to seven normal berries in the middle section (Figure 3). These berries weighed 7-8 g. All the other berries were much smaller (1.0-1.5 g) and contained no seeds. Maturity defined by the measurement of solid soluble dry matter with a refractometer was reached one or two days earlier on star flowered vines.

Molecular analysis

Of thirty RAPD primers used, only four (K1, K5, RAPD5 and RAPD6) did not yield any amplification (Figure 4).



Figure 1. Star flowered Cardinal inflorescence breaking open from the top.



Figure 2. Cardinal calyx opening not only from the top but also from the bottom in the same inflorescence.

Evaluation over twenty-six primer using RAPD-PCR revealed that normal and star flower variant Cardinal had the same DNA profile resulting in no polymorphism. It was concluded that there are no genetic differences between the Cardinal vines that produce normal and star flowers based on the analysis performed here.

DISCUSSION

Star flower formation may or may not be more common because its detection requires a keen eye with the knowledge that it might exist in a given vineyard. This may be the reason for limited reports of its existence (Portele, 1883; Despeissis, 1921; Kozma, 1960; Pratt, 1971; Queiroz-Voltan, 1998; Longbottom et al., 2004, 2008).



Figure 3. Normal (left) and star flowered (right) Cardinal clusters with very few normally developed seeded berries.

This is the first report of star flower formation in the Çanakkale region, or anywhere in Turkey for that matter. Ağaoğlu (1969) reported rougher leaf surface with rounded with a slight tendency to less lobing in star flowered vines. Although we found that there were some differences in the leaves and flowers, detailed study here showed that few differences exist between both types of Cardinal vines. DNA analysis revealed no polymorphism. Longbottom et al. (2008) reported also no polymorphic differences between star and normal flowered Chardonnay vines.

On the other hand, fruit set was incredibly lower on star flowered vines. Flowers that were fertilized did not fully develop into normal seeded berries. Star flower formation in Cardinal appears to be a barrier to obtain good fruit set and berry development. This is in agreement with Portele (1883), Despeissis (1921), and Pratt (1971). Longbottom et al. (2008) stated that cool spring time conditions might play a role on the relationship between star flower and seedless berry development. However, there is a need for long period of observations in connection with the weather conditions to draw any conclusion, hence this warrants further research.

Longbottom et al. (2008) also suggested that star flowers and the subsequent formation of seedless berries may occur parthenocarpically. Is this the case for Cardinal? This needs further investigation.

Longbottom et al. (2004) reported that star flower formation in Canada Muscat reoccurred in the second generation of flowers as the result of double pruning, and that the vines had likely been producing star flowers for years before discovery. Here in our study, the phenomenon also took place in the second year in the same vine.

Longbottom et al. (2008) speculated that star flower might be the result of deviations to the normal molecular pathway for flower development in *Vitis* genus and it might provide us a tool for understanding flower biology

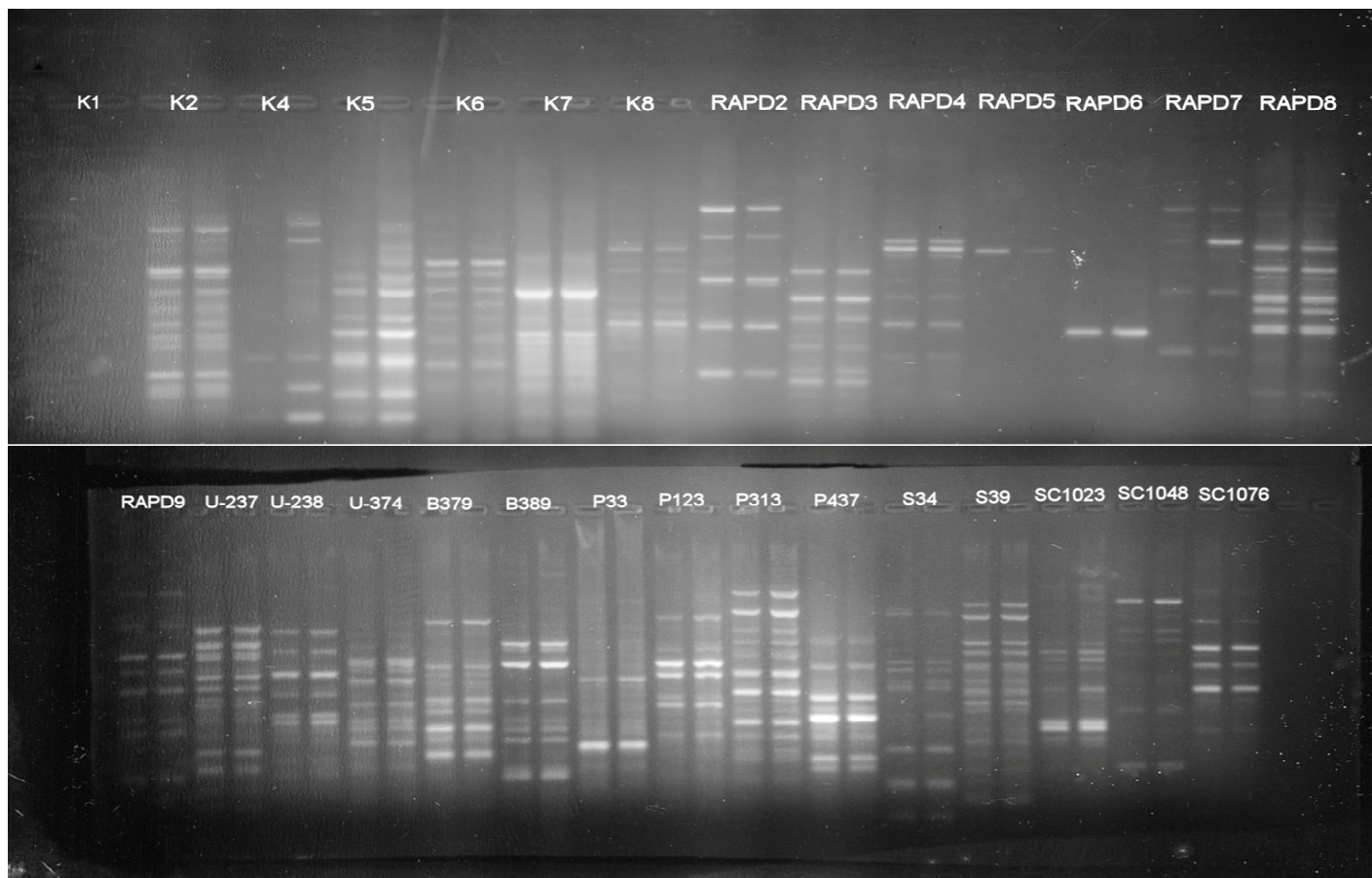


Figure 4. RAPD-PCR amplification results of star flowered (right column) and normal flowered (left column) Cardinal vines.

of grapevines. There are phylogenetic similarities between the genera *Vitis* and *Parthenocissus* (Gerrath and Posluszny, 1988; 1989). Gerrath and Posluszny (1989) established similarities in calyptral development and origins of petal and stamens between *Vitis* and *Parthenocissus*. However differences exist in the cells of the epidermis abaxial of the petals, being that in *Vitis* the cells are weaker in the region where abscission of the cells and consequent formation of the calyptra occurs. In Cardinal star-flowers, abscission layer might have been formed at the top due to the possibility that adaxial epidermis cells were weak here.

Conclusion

The observation of star flowers on different cultivars and in various parts of the world suggests that it may more commonly exist than formerly thought. Possible effects of low temperatures before and/or during flowering, cultivar dependence or bud mutations might have to be thoroughly investigated before discarding this phenomenon for the greater benefit of viticulture in the world.

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