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INFLORESCENCE AND FLOWER DIFFERENTIATION IN THE GRAPEVINE (*V. VINIFERA* L.) VARIETIES 'ŽILAVKA' AND 'BLATINA'

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Abstract

The different dynamics of flower differentiation i.e. flowering and the functional capacity of reproductive organs in grapevine must be studied as genotypic specificity under particular agroenvironmental conditions and brought to a level at which standardized biological control of fruiting is exercised in order to define sustainable production systems. Research on the functional morphology of the inflorescence and flower in the indigenous grapevine varieties 'Žilavka' and 'Blatina' as the major ones used for grape production in Bosnia and Herzegovina (B&H) was conducted in 2010-2011 through analysis of permanent histological slides. The morphological characteristics of the inflorescence, as observed through the presence and distribution of flowers, and the flowering dynamics of individual flowers in the inflorescence pave the way for the normal course of flowering and pollination in the studied varieties. During inflorescence development, the degree of flower congregation in the distal part of the inflorescence is defined as a varietal characteristic. The inflorescence in 'Žilavka' and 'Blatina' grapevines forms a cone-shaped panicle due to the different structure of side branches i.e. different morphology and structure of branching on the axes of simple botryose inflorescences along the inflorescence axis, when observed from the top down to the base. The sequence and dynamics of differentiation of the flower generative elements clearly indicate protandry in both varieties.

Key words*: grapevine, inflorescence, flower, characteristic*

INTRODUCTION

The grapevine inflorescence is a panicle belonging to compound racemose inflorescences with the axis growing forward relatively long at the tip forming side branches with flowers arranged in simple racemose inflorescences, and terminating in a flower (closed inflorescence). The grapevine inflorescence differentiates in the bud from the end of May until September in a single growing season (Mulins et al., 1992; Burić, 1981) and continues to develop in the spring of the following year. As stressed by Carmona et al. (2008), this fact is the main constraint to many studies aiming to explore all differentiation sequences and finally execute the genetic control of grapevine reproduction and yield. A more detailed analysis of flower differentiation in the inflorescence in accordance with the classification proposed by Mulins et al. (1992) has been provided by Caporali et al. (2003). 'Blatina' is an indigenous grapevine variety producing functionally female flowers that have a large effect on yield, which varies depending on the year. The production of a single-variety wine from this variety has not been a regular winemaking practice. As the 'Blatina' varietal wine does not exhibit long-lasting color stability, it is recommended to blend wines made from varieties used as pollenizers in plantings (Blesić, 2001). On the other hand, 'Žilavka' has been the leading grapevine variety in B&H for many years now (Mijatović, 1988; Tarailo, 1991). As estimated by Tarailo and Kovačina (1983), the important role 'Žilavka' plays in grapevine production and its heterogeneity in cluster and berry appearance make it possible to select a range of genotypes. However, when analyzing the genetic variability of 'Žilavka' by the AFLP technique Tomić (2009) determined the genetic profile characteristic of 'Žilavka' at 14 microsatellite loci and found no genetic background for the selection of 'Žilavka' clones or new genotypes with improved varietal

properties. The objective of this study was to examine the functional morphology of the inflorescence and flower in 'Žilavka' and 'Blatina' grapevines as the basis of success in micro- and macrosporogenesis.

MATERIALS AND METHODS

Anatomical, morphological and cytohistological characteristics of reproductive organs were evaluated in two major indigenous varieties in B&H - 'Blatina' and 'Žilavka'. Part of the experimental research and sampling for the anatomical, morphological and cytohistological analyses of reproductive organs were conducted in a commercial vineyard of the Aluminium Plant, Mostar (southeastern B&H). The varieties were grafted on Kober 5BB rootstock, with vines trained to the Moser cordon system and subjected to short pruning. Standard agricultural and viticultural practices were used, which along with growing conditions (modified Mediterranean climate) ensured steady yields. Samples for the preparation of histological slides were collected from initial cluster emergence until fertilization. The cytohistological analysis of the reproductive organs involved preparation of permanent histological slides. Sampling for the preparation of histological slides was performed during shoot and inflorescence growth and at early flowering and pollination stages. Inflorescences were sampled every two days between 9 and 10:30 a. m. from 27 April until 25 May in 2009 and 2010. In the field, immediately after removal from the shoots, the inflorescences were placed into bottles and fixed in Navashin's fixative. The fixed paraffin-embedded specimens were sectioned using a microtome at a thickness of 9 *ηm*. The differential staining of the histological sections was performed by Delafield's hematoxylin. The sections were analyzed under an Olympus/DP/SOFT light microscope. Images were documented using an Olympus/DP camera and edited by the Image Analyzer software.

RESULTS AND DISCUSSIONS

During inflorescence development, the degree of flower congregation in the distal part of the inflorescence is defined as a varietal characteristic. This aggregation and grouping of flowers in simple botryose inflorescences on side branches in the basal half of the panicle is the result of the progressive shortening of the internodes on the side branches of the inflorescence. Reduction in internode length on side branches which carry simple botryose inflorescences is a variety-specific morphological character in ampelography as it results in variable compactness of the infructescence (the arrangement of berries in the cluster), and at preflowering it can be associated with the mechanical pressure on petals, that is, this compactness among flowers can have a role towards flower caps in terms of flower opening-cap fall.

Figure 1. Structural analysis of side branches of an inflorescence histologically (left) and morphologically (right) showing flowers grouped in simple clusters in the following arrangement - the apical part of the side branch terminating in three flowers which have bract rudiments at their base; remaining flowers grouped in simple botryose - racemose inflorescences whose number and distribution on the shortened to compact axes of the side branches of the inflorescence can be observed primarily through bract presence

The morphological characteristics of the inflorescence cannot be directly associated with functional morphology as the presence and distribution of flowers and the flowering dynamics of individual flowers in the inflorescence cannot be associated with pollination or fruit set. The first branch of the inflorescence closest

to the base is often quite distant from the other branches and constitutes a distinct part of the inflorescence. During further growth and development, this branch of the inflorescence can develop into a small cluster called a lateral cluster or a wing, which can reach the length of the main inflorescence in some varieties. This inflorescence character has been established in 'Žilavka' grapevine and is considered its varietal characteristic as regards the ampelographic characterization of the cluster (Tarailo and Kovačina, 1983; Mijatović, 1988; Tarailo, 1991). The differentiation of flower primordia was observed dynamically from the microstage at which the emergence of inflorescence axes on the shoots was visible until fully formed flowers (Carmona et al., 2008). The research performed at this level of observation showed no anatomical, morphological or histological differences in the differentiation of individual flowers in the studied varieties. The difference in the differentiation dynamics between the varieties and among the inflorescences differently positioned on the shoots at this point of the research was analytically generalized so that more extensive histological and cytological analyses of individual flowers could be executed for the evaluation of their individual elements. The flower of the grapevine is pentagonal, composed of 5 sepals arranged along the margin of the receptacle. As the sepals are poorly developed, they can be considered rudimentary. The inside of the calyx holds five petals which are fused along their entire length. There are 5 stamens, completely free and practically located in the axils of the petals. Each stamen consists of an anther supported by a filament. The pistil occupies the center of the flower, and comprises the ovary, stigma and style. The ovary is bilocular, having two carpels, morphologically two chambers, each containing two ovules. The histological analysis of flower primordia becoming visible as a small pinhead shows the differentiation of sepal primordia enclosing the less conspicuous petal primordia, which form a roof over dome-shaped anther initials (Figure 2a). At the microstage when the petals surpass the sepals by their whole length, all anther primordia elements differentiate, and initial carpel differentiation is observed (Figure 2b).

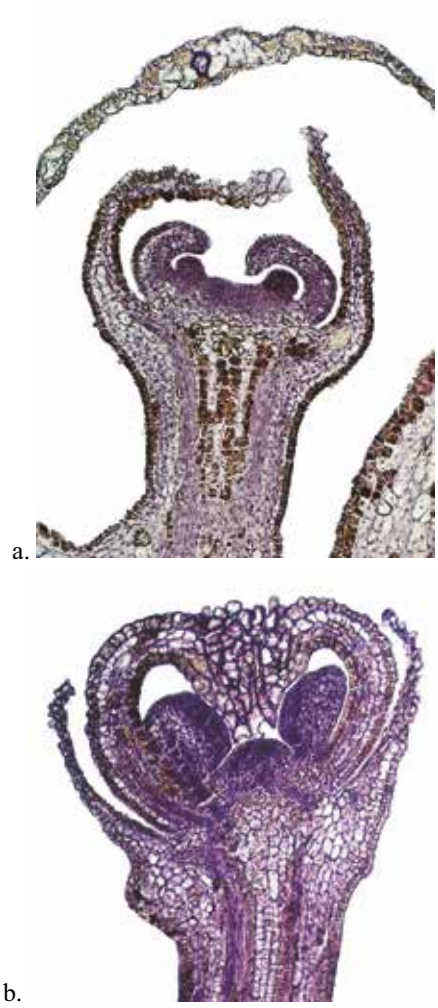

Figure 2. Microstages of flower differentiation in 'Blatina': a) sepals, petals and anther primordia differentiated on the flower axis; b) microstages of the differentiation dynamics of flower elements observed in relation to the sepals whose development is arrested as early as the beginning of flower primordium development, due to which they practically remain rudimentary. The petals fuse at the tips and cover anther initials, keeping them leaning against the stigma of the ovary

The differentiation sequence and dynamics of the flower generative elements clearly indicate that both varieties are protandrous. Protandry is fully visible in the histological sections of the anthers and ovaries in the same flower initials (Figure 3).

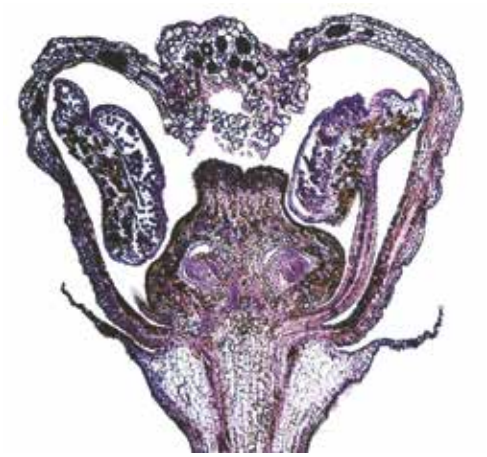

Figure 3. Microstages of flower differentiation in 'Blatina'. At this microstage of flower primordium development, the final stages of microsporogenesis and initial stages of macrosporogenesis are observed

The results on the dynamics of flower differentiation in the inflorescences of 'Žilavka' and 'Blatina' grapevines, as observed on the histological slides, are in complete agreement with the flower differentiation dynamics reported by Caporali et al. (2003) based on the classification proposed by Mulins et al. (1992). The cross-section through the center of the flower shows the position of the anthers relative to the petals and the manner in which the petals fuse to form the flower cap in the studied varieties. Minor differences are observed in the length of the fusion zone between the varieties (the fusion zone being somewhat longer in 'Žilavka' than in 'Blatina'), which can affect the rate of cap detachment during bloom (Figure 4 a, b). The center of the flower is occupied by the pistil made up of two fused carpels - microsporophylls. The ovary is bilocular - it consists of two chambers, each containing two ovules.

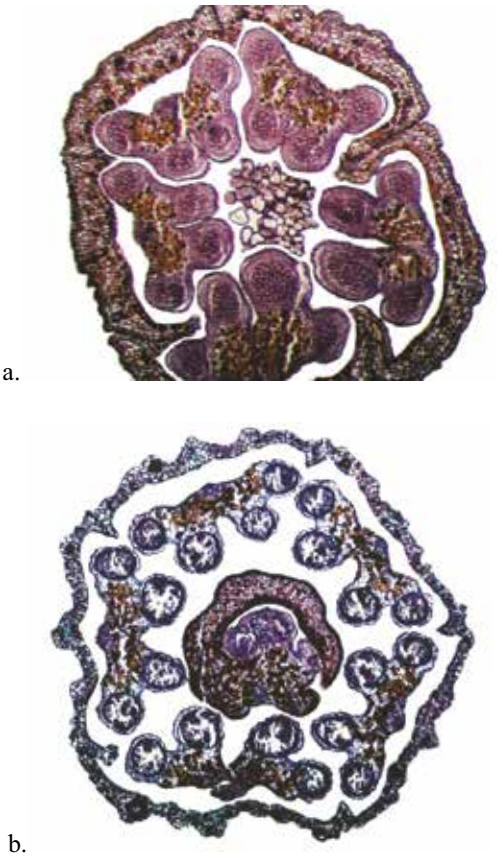

Figure 4. Histological cross-sections of flower initials in 'Žilavka' (a) and 'Blatina' (b) clearly showing the petal fusion zone

The partition between the chambers is formed by the bending and curving of the carpel margins inwards towards the interior of the ovary. In this way, the carpels fuse laterally, dividing the ovary into two chambers. All ovules are initiated perpendicular to the margin, and then nucellar and integumentary cells grow and develop at different rates, giving rise to anatropous ovules with the micropyle facing the base of the ovary. In this way, four anatropous ovules in marginal placentation are formed in the ovary of each variety (Figures 5 and 6). The central part of the floral structure is occupied by the ovary composed of two chambers, each holding two ovules.

Figure 5. Histological representation of the cross-section of the bottom third of an individual flower, in 'Žilavka'. The cross-section displays the formed ovules at the integument differentiation stage. Cross-sections of the filaments are visible in the zone between the ovary and the petals. The cross-section of the filament in 'Žilavka' clearly shows epidermal and parenchyma cells located at the periphery of the filament and somewhat smaller central cells of the vascular tissue). This anatomical structure of the filament is typical of bisexual flowers in the grapevine

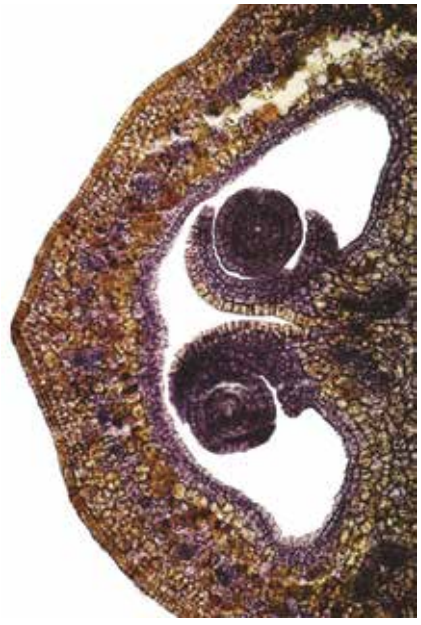

Figure 6. Histological representation of the morphological and anatomical structure of a portion of the ovary in 'Žilavka'. The ovule develops in the interior of the ovary and has marginal placentation. Later on, the ovule develops into a seed. The cross-section shows the ovule the size of a small bulge covered by the integument

The analysis of inflorescence and flower differentiation in the tested varieties is consistent with the findings of Caporali et al. (2003) who reported that functional differences between the (male and female) flowers are commonly recorded at this stage, when no style and stigma formation occurs on the pistil of male flowers. Due to these morphological changes, the pistils of male and female flowers are quite different at the next stage of development, whereas their stamens still remain very similar in appearance.

CONCLUSIONS

The inflorescence in 'Žilavka' and 'Blatina' grapevines forms a cone-shaped panicle due to the different structure of side branches i.e. different morphology and structure of branching on the axes of simple botryose inflorescences along the inflorescence axis, when observed from the top down to the base. The degree of flower congregation in the distal part of the inflorescence can be defined as a varietal characteristic. This aggregation and grouping of flowers of simple botryose inflorescences on side branches in the basal half of the panicle is the result of the progressive shortening of internodes on the side branches of the inflorescence. The histological analysis of the flower primordium visible as a small pinhead at the morphological stage shows differentiated primordia of sepals enclosing the less conspicuous primordia of petals, which form a roof over dome-shaped anther initials. The differentiation sequence and dynamics of the flower generative elements clearly show that both varieties are protandrous. All inflorescence and flower differentiation processes in the tested varieties take their normal course, with no anomalies potentially adversely affecting pollination and fertilization found at this level of observation.

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