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MALFORMATIONS OF REPRODUCTIVE ORGANS IN WALNUT (*JUGLANS REGIA* L.)

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Abstract

In this research, which was conducted in *native walnut population*, during 2000-2011 years in the region of Kraljevo (central Serbia), more than 2000 *walnut* seedling trees were observed. In some trees unusual phenomena in the structure and function of reproductive organs were expressed, such as: second and successive flowering, mixed inflorescences with female flowers at its base and male flowers at the top, hermaphrodite flowers, “V” shaped catkins, spurs only with catkins, flowers and fruits fusion, irregular shell and kernel segmentation and *incomplete involucres.* Certain of these malformations occur in some trees every year, while in some cases they represent a sporadic phenomenon, probably caused by environmental factors. In this paper all these phenomena are described in detail and documented by photographs.

Keywords: *walnut, reproductive organs, malformations*.

Introduction

Walnut growing has a long tradition in Serbia, where the number of bearing trees is around two million. The soil and climatic conditions in most regions are suitable for walnut cultivation, and walnut trees are found in all fruit-growing regions. It has been propagated from seed for centuries, resulting in a heterogeneous population in which each tree is a different genotype (Cerovic et al., 2010).

Walnut (*Juglans regia* L.) is monoecious plant, and its male and female flowers are born separately on the tree. It normally develop unisexual inflorescences (Hickey and King, 1981). The male flowers are densely packed on catkins, which hang from the tree in the spring. Each catkin has up to 40 sessile petalless florets, each with numerous stamens. The immature naked catkin buds first appear in leaf axils in late summer and persist over winter, maturing in the spring in the axils of leaf scars on wood from the previous season. Female flowers are borne on the current season’s growth in spikes of two to five flowers. Flowers are typically produced on the tips of terminal shoots shortly after leaves emerge. The female flower’s entire basal portion is enclosed within a hairy involucres formed by the fusion of several accessory flower parts (McGranahan and Leslie, 2009). Inside the involucres is the pistil, which has a swollen base (the ovary), and a short style, with a forked stigma with two feathery stigmatic lobes. The ovary is surrounded by the ovary wall. Attached to the ovary is a single ovule enclosed by a single integument (Polito, 1998; Pinney et al., 1998). The fruit is a nut enclosed in an indehiscent, thick husk. The fertilized egg is zygotic tissue and becomes the embryo and later the edible kernel. The kernel consists of two fleshy cotyledons and an embryonic axis (Janick and Paull, 2008).

Abnormal flowers have been recognized as curiosities by botanists for more than two thousand years (Meyerowitz et al., 1989). It can be induced by several environmental

adversities and by genetic factors. The nowledge of genetic relationships among walnut genotypes and their reproductive characteristics are really useful in walnut cross-breeding programs (Cosmulescu and Botu, 2012). The objective of this paper is to present various anomalies observed on the reproductive organs of walnut *during the eleven-years period of investigation* of the native walnut population in the Kraljevo area.

Materials and methods

The study was conducted in *native walnut population in several localities in the region* of Kraljevo (central Serbia), during the period 2000 - 2011. More than 2000 *walnut* seedling trees were observed. The main objective of this study was to examine the phenological and reproductive traits of walnut trees in spontaneous habitats and selection of interesting genotypes. The study included *sampling and observation of collected plant material.* During this study various anomalies of the walnut reproductive organs *have been* identified, which has *been* photographed and described.

Results and discussion

Apomixis

Apomixis in flowering plants is defined as the asexual formation of a seed from the maternal tissues of the ovule, avoiding the processes of meiosis and fertilization, leading to embryo development. Therefore, this embryo has a genetic constitution identical to that of the female parent (Bicknell and Koltunow, 2004). Apomixis is present in walnut. *Rate of apomictic fruits* in walnut differs from year to year and depends on climate and genotype (Asadian and Pieber, 2005). The mechanism of apomixis in walnut has been reported as adventitious embryony (Valdiviesso, 1990), apospory (Terziiski and Stefanova, 1990), or diplospory (Sartorius and Stosser, 1991). We identified apomixis in several walnut genotypes during the period of examination. In all these cases we have recognized the apospory (Fig. 1-a).

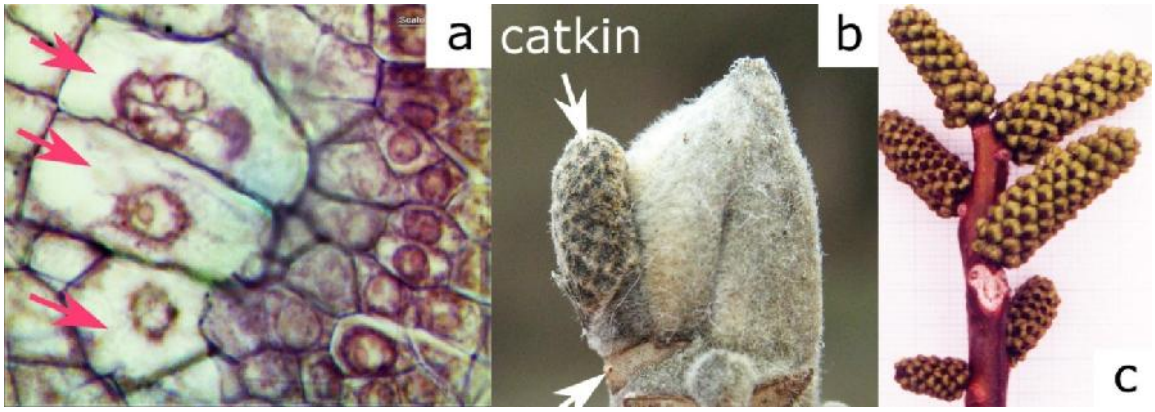

Figure 1: a) *aposporic embryo* sacs in the walnut nucelus; b) catkin formed in the axill of the terminal bud scale; c) “male spur”.

Catkin development from the terminal growing point of the shoots

Male flowers of walnut are grouped in catkins, which develop laterally on the previous season’s growth (Polito, 1985). On the adult trees, monocyclic shoots (that are formed by the first growth flush in spring) are usually female, flowering in the terminal position (Solar and Štampar, 2003). In some genotypes catkins are formed in the terminal position of the shoots.

These genotypes are prone to form “male spurs”, which carry almost no other buds than catkins. Sometimes, a catkin is also formed in the axill of the terminal bud scales (Fig. 1-b, c).

Occurrence of the “V” shaped catkins

In the year 2002 almost all catkins from one solitary walnut tree were “V” shaped. We did not detect reason of that phenomenon, but it is certain that it was induced by environmental factors (Fig. 2-a).

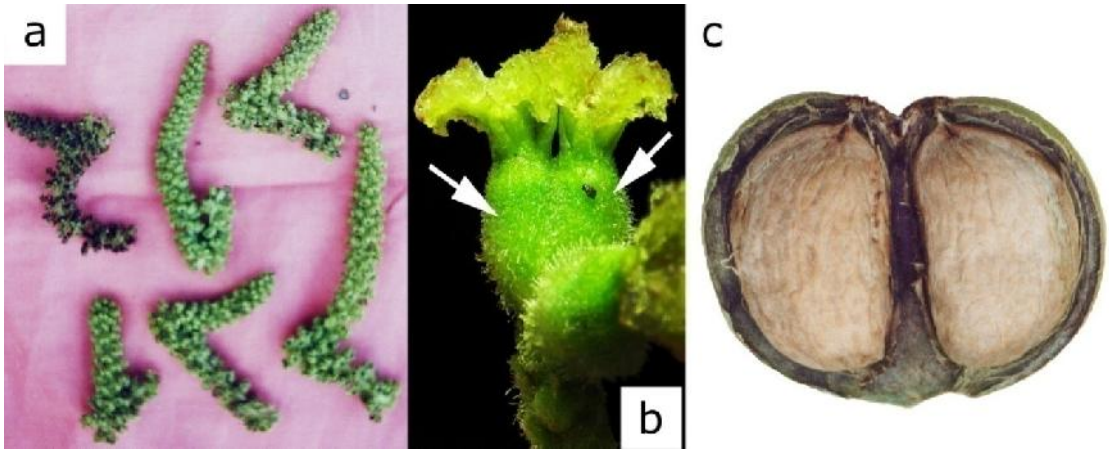

Figure 2: a) “V” shaped catkins; b) female flower with double pistils; c) twin fruits developed from a female flower with double pistils.

Doubling of pistils

Surányi (1979) studied floral anomalies of several *Prunus* species and found on average, 1,7% twin pistils. High summer temperatures at the time of flower bud differentiation are believed to cause forming of double pistils, resulting in many double or malformed fruits at harvest (Micke et al., 1983). Besides the normal flowers, with two stigmas, Kholdorov **(**1975) noticed the walnut flowers with three stigmas in some genotypes. In our examination, we found several examples of double pistils (Fig. 2 - b, c), but this abnormality was not common.

Figure 3 – second flowering in walnut: a) hermaphrodite inflorescence; b) female inflorescence; c) hermaphrodite flowers; d) hermaphrodite inflorescence with mature fruits.

Second flowering and hermaphroditizm

In some walnut trees, after the normal flowering, secondary flowering waves are also identified during a growing season. Walnut normally develops unisexual inflorescences, but *in case of secondary flowering* inflorescences may carry male, female and hermaphrodite flowers. Female inflorescences are grouped at the top of relatively small shoots with very short internodes (Fig. 3 - a). *On the hermaphrodite inflorescences* female flowers are placed at its base and male flowers at the top (Fig. 3 - a, d). Sometimes on the mixed inflorescences partially developed hermaphrodite flowers were placed, with a pistil in the centre and the stamens around it (Fig 1 - c). Similar phenomena have been described by Germain et al. (1997) and Breton et al. (2004) in some walnut seedlings originating from central Asia.

Abnormalities in fruit development

As the fruit matures, the involucrum and fused sepals develop into the husk (hull), and the shell is formed from the ovary wall. Walnut fruit contain two kernels separated by a thin, papery central plate, extending from the inner layer of the shell. Two primary (major) septa grow inwards from the point of union of the two carpels and unite the lower part of the fruit to a varying degree. The major septum is in the plane perpendicular to the suture lines. Two secondary (minor) septa may also develop in the lower part of the endocarp, causing the seed to become four-lobed at its base. The minor septum lies in the plane of the shell sutures. The endocarp dehisces along the two prominent midribs situated opposite the two cotyledons. *In this study we noted three genotypes which form abnormal fruits. Two of them had the fruits with irregularly formed sutures on the shell and septa (nuts with ½, 1, 2, 3, 4, and 5 sutures) (Fig. 4-b). The third genotype formed* fruits with *incomplete involucrum,* below which the sepals are visible *(Fig. 4-a).*

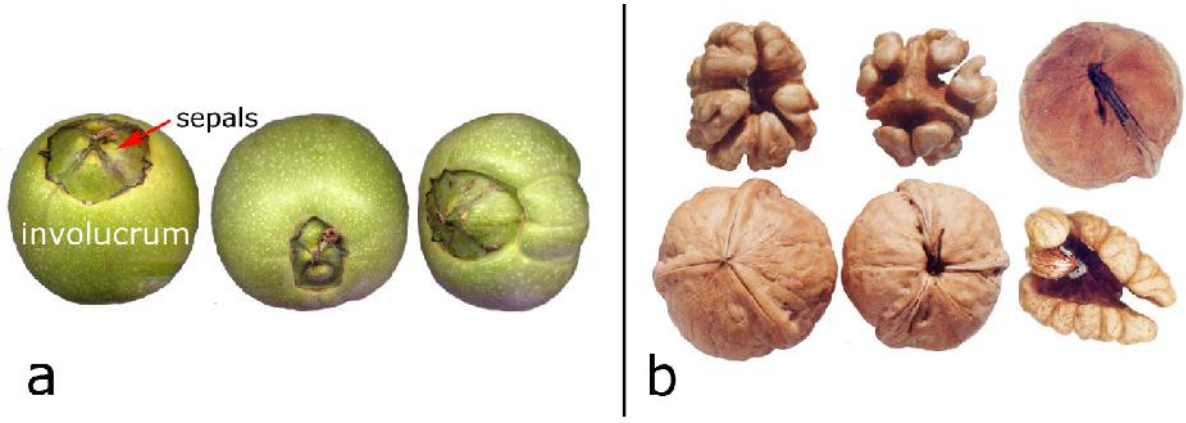

Figure 4: a) fruits with *incomplete involucrum; b) fruits with irregularly formed sutures on the shell and kernels.*

Conclusion

During the period 2000-2011 in the *native walnut population* in the region of Kraljevo unusual phenomena in the structure and function of reproductive organs were expressed such as: second and successive flowering, mixed inflorescences with female flowers at its base and

male flowers at the top, hermaphrodite flowers, “V” shaped catkins, spurs only with catkins, flowers and fruits fusion, irregular shell and kernel segmentation and *incomplete involucres.* Certain of these malformations occur in some trees every year, while in some cases they represent a sporadic phenomenon, probably caused by environmental factors. In the case of the heritable abnormalities it is possible to study gene interactions and gene dosage effects when they influence the degree of abnormality expressed by the plant.

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