Early bearing genotypes of walnut: a suitable material for breeding and high density orchards

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Abstract
Walnut (*Juglans regia*) is a non-precocious bearing and vigorous tree. Standard seedlings of walnut have long juvenility and are hard to propagate by usual propagation methods like cutting, layering and somehow grafting. There is an excellent genetic diversity among *Juglans regia* populations from China to central and west Asia including Iran. Among the populations there are some genotypes of walnut that are precocious and have short juvenility phase. These genotypes usually have cluster bearing flowering habit, low vigor and good rooting ability that make them suitable for easy propagation by cutting and stool layering. Most of these genotypes have basitonic phenotype in nature. Such dwarf genotypes which have ability to induce precocity in scion can be used not only as a rootstock in walnut breeding programs to shorten the vegetative adult phase of the scion and reduce duration and costs of a breeding program, but also have potential for establishing high density orchards. Precocity trait also has a high heritability in walnut. Low vigor genotypes show the highest proliferation rate and smaller shoot size, lower callus formation and higher rooting percentage in vitro. Moreover, these genotypes showed in vitro flowering on microshoots. Therefore, these genotypes of walnut are an ideal material for molecular genetic investigations for breeders and geneticists to find the genes involved in precocity, growth, flowering habit, easy rooting and other valuable traits in walnut. In this article we review the researches done about these genotypes.

Keywords: *Juglans*, precocity, propagation, rooting, grafting

INTRODUCTION
Dwarf rootstocks are used to control vigor and fruit quality in many fruit trees including apple, pear, citrus, cherry, etc. (Webster, 1995). Dwarfness and induction of precocity (early bearing) will be attractive for the walnut growers all around the world particularly in Asia, because most of the trees are hand harvested, with high expenses, and many workers fall down from the trees and injure or die every year. For these reasons, many walnut growers are now interested in shifting to high density planting systems in walnut (McGranahan and Leslie, 1988; Ramos et al., 2001).

Reducing tree size using genetically dwarf rootstocks is a key component for high density orchard systems. For the first time, Germain et al. (1997) reported existing of these genotypes in central Asia. Vahdati et al. (2009) reported that precocious and low vigor walnut genotypes are frequently found in some seed source plantations of walnut in Iran.

Some investigations have been carried out on these genotypes and results appeared that there are some differences between these and standard genotypes in vigor, precocity, rooting, etc. According to the current investigations, these walnut populations are not only extremely precocious, in comparison to other cultivars which flower at the same time of the year, but also can yield elite cultivars with genes for the dwarfing property (Wang et al., 2013).

VIGOR AND HEIGHT
Rezaee et al. (2006) reported that seedling size and precocity in Persian walnut vary
depending on the seed source used in nurseries. This variation allows breeders to select dwarf and precocious seedlings. Moreover, Rezaee et al. (2006) reported that shortest seedlings are generally observed in the offspring of low-vigor and cluster-bearing families. Dwarf genotypes of walnut may have a higher proportion of homozygous loci that are responsible for reduced vigor of growth, which consequently produce a higher proportion of dwarf offspring. Vahdati et al. (2008) reported that there are endogenous hormonal and histological differences between high vigor and low vigor seedlings in walnut. Rezaee et al. (2006) calculate correlation between some morphological traits among walnut populations. According to the results, there are moderate to high heritability (0.39-0.88) between-family, implying that the majority of phenotypic variations are under additive genetic control. Seedling height is positively correlated with seedling diameter \((r=0.87)\), number of nodes \((r=0.86)\), and length of the internodes \((r=0.89)\), indicating that measuring only seedling height is adequate to identify families that produce dwarf seedlings. The single gene system may also be proportional for dwarf and precocious walnut trees; as the most extreme dwarf offspring were obtained from dwarf and cluster-bearing parents. Wang et al. (2013) reported that developing dwarf walnut cultivars by selecting dwarf individuals from a seedling population of second-bearing walnuts is not only feasible, but also is an efficient method for developing new cultivars with this useful trait.

ROOTING ABILITY

Walnut is known as a "difficult-to-root" genus among fruit trees. Vahdati et al. (2008) confirmed that there is variability between walnut populations in rooting ability. Variability between genotypes in rooting was also reported in other woody plants like peach (Tsiouridis et al., 2003), and grapevine (Peros et al., 1998). Results appear that rooting ability of low-vigor seedlings by stool layering is better than in semi-vigorous and high-vigorous seedlings (Vahdati et al., 2008). The number of seedlings which showed rooting ability in the low vigor seedlings was approximately 2.5-times greater than in the high vigor seedlings. Rooting ability of high vigor seedlings might be impaired by higher amount of gibberellins, and/or as a result of greater lignification, wood density and rigidity of the sclerenchymal ring. Therefore, by reverse selection in favor of low vigor seedlings, it is not only possible to improve rooting ability, but also to exploit their extensive benefits for increasing fruit yield in a high-density orchard systems by vegetative propagation of dwarf rootstock. It is also possible to increase the frequency of favorable alleles contributing to rooting ability by inter-crossing or self-crossing of easy-to-root clones for advanced quantitative genetic analysis and QTL mapping of loci that control rooting.

DWARFING EFFECT ON SCION

Rootstock breeders used dwarf trees as the graft recipients of vigorous cultivars, and used the same dwarf or dwarfing cultivars combinations in high density production orchards. The influence of rootstocks on scion architecture can also encompass changes in the number of lateral branches (feathers) and their angle with the trunk (Fazio and Robinson, 2008). These rootstock-mediated changes seem to be physiologically different from the inherently dwarf or clonemar scions and other heritable architecture types in apple (Conner et al., 1998; Hemmat et al., 1997). According to the results reported by Mahmoudi (2011), cluster-bearing walnut rootstocks decreased scion height, length of internodes of the scion shoots, and increased formation of lateral shoot, compared to the standard ones. Cluster-bearing rootstocks also enhanced precocity in comparison to the standard rootstocks. However, Rezaee et al. (2008) reported that growth rate of scions 45 days after grafting was affected significantly by the seedlings vigor. Also, the rate of scion growth (height) was reduced significantly on low-vigor seedlings at the end of the growing season (Rezaee et al., 2008). This could be attributed to their smaller root system and lower growth rate, as explained in other fruit trees. Therefore, influence of seedling rootstocks obtained from a cluster-bearing walnut tree on control of walnut tree size will be confirmed by grafting in the field conditions.
DWARF CULTIVARS

Recently, some dwarf and early bearing walnut cultivars as scion were released through a selection and breeding program during 1998-2010 in China (Wang et al., 2013). The successful selection of dwarf walnut cultivars demonstrates that scientific application and the objectivity of the theory that the height of walnut trees is closely related to the precocity trait. Moreover, four elite early mature walnut trees were selected, which had high and stable yield, large fruit, thin shell and dwarf type, based on the tree body traits, fruit characters and fruit number in China (Wang et al., 2010).

IN VITRO PROPAGATION

Proliferation and rooting ability of some low vigor and early mature seedlings of Persian walnut were compared with those of semi- and high vigor seedlings in in vitro condition. Vahdati et al. (2009) reported that the low vigor genotypes have the highest proliferation rate and smaller shoot size, lower callus formation and higher rooting percentage. Moreover, these genotypes showed in vitro flowering on micro-shoots, which are consistent with the field observations (Breton et al., 2004; Vahdati et al., 2009). These results proved the consistency of low vigor, precocity, basitonic growth tendency and easy rooting of dwarf and precocious genotypes under in vitro condition.

FLOWERING HABIT

Two types of flowering habit were observed in early mature walnut genotypes: normal flowers and flowers in clusters. Bisexual inflorescences and hermaphrodite flowers are regularly observed among the cluster bearing mother trees cultivated in the field (Germain et al., 1997). More investigations on the whole catkins and individual florets indicated that each of the florets had both abundant yellowish dusty pollen and a well exposed ovary with 2 or 3 feathery stigma at the center of florets. At this time, the normal process of flowering was completely finished and the normal nuts on the shoot tips were in their fast stage of growth. These modified sex catkins reached their final length of about 10 cm in late spring.

After self-pollination, fertilized ovaries begin to develop nuts from the base of catkins to the apex. By early summer, each catkin had many small nuts, which looked like a berry cluster. At the same time, size of the normal nuts were about 5 times larger than the above mentioned ones. Of course, some of the catkin's nuts, mainly those at the tip of catkins, were dropped and only 3 or 5 of them remained intact until the harvesting time. Also, the maturation of these nuts was about 1 week later than normal nuts. It should be noted that this phenomenon is completely different from lateral bearing habit (R. Rezaee, unpublished data and our observation).

Early flowering is responsible for increasing branching of early mature trees, which are generally smaller and bushier than other genotypes. The early flower genotypes also exhibits early bud break and high susceptibility to the walnut blight caused by Xanthomonas campestris pv. juglandis (Germain et al., 1997).

PRECOCITY

Early-bearing walnuts begin to flower 1-3 years after planting, in nursery, while the late-bearing walnut has a long juvenility period, so that under field conditions, first flowering usually occurs when trees are 5-9 years old (Sabatier and Barthelemy, 2001) and occasionally even more than 11 years old. Therefore, early-bearing walnuts were thought to be a rare resource in woody plant’s precocity, juvenility and flowering mechanism study and would play a potential role in shortening juvenile phase of perennial woody plants, as well as in cultivars breeding and modification by genetic engineering through walnut improving programs. Thereafter, numerous inflorescences arising from lateral buds lead to large fruit sets, and intense secondary flowering waves take place during the same growing season. The natural flowering capacities of cluster bearing walnut trees provide a valuable resource for research and breeding projects aimed to increase fruit production or shorten juvenility time in trees. In order to accelerate the breeding speed, it is useful to predict some properties of trees in the adult stage using indicator properties and character traits of
juvenile trees. This is frequently done to increase the efficiency of tree cultivar breeding.

**MOLECULAR MARKERS STUDY**

Ye et al. (2012) reported a SCAR marker related to early-bearing trait in maternal parents and F1 generation of walnut, which was 762 bp. Also in this study it appeared that the early-bearing trait has high heritability in walnut. A study for establishing fingerprint and phylogenetic relationship was performed between six walnut dwarf cultivars and genetic similarities (GS) calculated by two polymorph ISSR markers in China. The GS among cultivars ranged from 0.54 to 0.94. The eight walnut cultivars clustered into two groups. The female parent of 343, dwarf cultivar of ‘Xinwen724’ and ‘Xinwen908’ were in the first group; and the second group included female parent of ‘Xinzaofeng’, dwarf cultivar of ‘Xinwen609’, ‘Xinwen915’, ‘Xinwen916’ and ‘Xinwen917’ (Wang et al., 2011).

**CONCLUSIONS**

Early bearing walnut genotypes have been studied in last 30 years. The results of different studies reveal that there are some benefits such as good rooting ability, precocity, cluster bearing, low vigor or semi-dwarf growth habit among these genotypes. Early-bearing walnuts are rare resources which have many valuable traits such as precocity, dwarfness, low juvenility and high yield. Genetic and molecular study of these genotypes could identify the genes involved in these traits. Also elite dwarf and precocious rootstocks may develop by conducting a rootstock breeding program using these genotypes.

**Literature cited**


