



A promising fruit: Cherry laurel (*Prunus Laurocerasus* L.) and steps on breeding

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ABSTRACT

Cherry laurel (*Prunus laurocerasus* L.), is a fruit native to the regions bordering the Black Sea in Southwestern Asia and Southeastern Europe and widely spread out in the North part of Turkey. There are many cultivars which show different characteristics in Turkey and germplasm provides a rich parental material for crossing opportunities. The cherry laurel tree has pleasant fruits when fully ripe and also is a valuable fruit for industrial uses. Production of this fruit has increased over time and evaluation possibilities are varied. The detailed information about characteristics and nutritional value of cherry laurel was obtained from earlier studies and served for breeding of new cultivars. Studies on breeding of this species are focused on selection of superior types, resistance to disease, environmental adaptability, and molecular mapping of these types. This review aims to create a perspective to results of undergoing studies and discusses an overview of breeding opportunities of cherry laurel.

Keywords: *prunus laurocerasus*, breeding, selection, molecular studies, biological studies, propagation

Introduction

Cherry laurel (*Prunus laurocerasus* L.) is an evergreen shrub or small tree up to 6 m; the leaves are dark green 5-15 cm in length and 4-10 cm in width and is naturally grown around the Black Sea Region, especially in North and South Anatolia (Kolayli et al. 2003). Cherry laurel has the chromosome number $2n = 22x = 176$ (Contreras and Meneghelli 2013). Seeds of cherry laurel easily are spreading by birds which have led to concern regarding its increasing potential. The fruit is a small cherry, turning red to black when ripe. It is mostly consumed as fresh fruit in local markets, and the fruit of the cherry laurel is used in making jam, pickle, and cake; it is also eaten as dried and become more popular in the recent years (Sulusoglu and Cavusoglu 2011a). As a result of increasing market value, the demand for the fruit by producers and processing industry has increased which is an opportunity for the developing commercial cultivars for cherry laurel. Tapping germplasm resources to improve cultivated plants

depends on introducing natural variability through traditional and biotechnological breeding methods (Bridgen 1994). Selection is basic of the fruit breeding and is the corner-stone upon. Studies have focused on selection of high productive cultivars of cherry laurel and determining of characteristics of the selected types, as well as propagation methods economically too. Herewith follows a brief overview of the main topics regarding cherry laurel breeding.

The first step of the breeding studies Selection of superior cultivars

Efforts of characterization studies of cherry laurel can be traced into the late of 1900s, in which the incredible advances have been achieved for the last 20 years in terms of cultivar development. Studies have been conducted to determine promising cherry laurel types in the different regions of Turkey and trees with different ages at different locations under various growing conditions were identified and some of their pomological and chemical traits were determined

(Table 1). Studies on the selection of superior types have provided valuable information to help select the appropriate types in breeding programs for targeted cultivar development.

The first study reported in the literature was conducted by Özbek (1952) in Giresun forest area. Özbek made a travel in the Black Sea Region and investigated tree and fruit characteristics of this specie. Especially 'Fındık' cultivar grow in Giresun was evaluated briefly. Other studies were conducted in Trabzon, Rize, Ordu, Samsun, Sakarya and Kocaeli cities of Turkey (İslam 1996; Karadeniz and Kalkışım 1996; İslam and Odabas 1996; Bostan and İslam 2003; Akbulut et al. 2007; İslam and Vardal 2009; Beyhan 2010; Sulusoglu 2011b; Macit and Demirsoy 2012; İslam and Deligöz 2012) that all of the sites has Black Sea Region characterized by clay-loam soil texture with temperate climate in which winters are not very cold and summers are warm-hot with rare spring frosts.

Fruit quality in general has been a driving force in selection studies efforts. Fruit size and weight is important criteria to select of commercial types. The acceptable cherry laurel is sweet, with little astringent taste and rich color when ripe (Sulusoglu and Cavusoglu 2011a). The fruit has a seed inside and flesh to seed ratio is important criteria for the selection of types to industrial use in the future studies.

The modified weight rank method with rates from satisfactory to undesirable with a relative scale for every character (Macit, 2008; Beyhan, 2010; Sulusoglu, 2011b; İslam and Deligöz 2012; Macit

and Demirsoy, 2012) has been used to determine the superior types. These selection criteria used for cherry laurel is tree productivity criteria (yield per trunk diameter area, yield per tree, fruit number per cluster, fruit harvesting time), fruit characteristics (fruit weight, width or length of fruit, fruit firmness, flesh/seed ratio, appearance of fruit, fruit uniformity) and fruit chemical characteristics (soluble solid contents, astringency, fruit taste, titratable acidity).

Pomological studies indicated that genotypes selected as promising had showed variability in the different areas of Turkey. Most of the studies were carried out in the different ecological conditions and under different ages of tree that were managed under different cultural conditions. All of these factors negatively affected the productivity of the trees and fruit quality. Only one experiment was conducted under the controlled conditions, in Samsun (Macit and Demirsoy 2012). Selected cherry laurel types from different place of the Black Sea region were planted in an orchard in Samsun and phenological and pomological analysis completed and some of these genotypes were suggested as a raw material for the breeding programs. Adaptation studies need to be organized in the other potential areas which are the second phase of the selection studies. Orchard performance of the selected types will give better results for selection of promise types as a cultivar.

Less known fruit species received much attention for their health benefit substances including antioxidants, total phenolics and fatty acids. Cherry laurel fruit serve as a good source of natural

Table 1. Pomological and chemical characteristics used in the selection of Cherry laurel types

Study area	Pomological and chemical character									Literature
	Cluster length (cm)	Fruit number/cluster	Fruit weight (g)	Fruit width (mm)	Soluble solids (%)	Titratable acidity	Flesh seed ratio	Fruit taste	Astringency	
Samsun	-	+	+	+	+	+	+	+	+	Macit and Demirsoy, (2012)
Ordu	+	-	-	+	+	+	-	+	+	İslam and Deligöz (2012)
Kocaeli	+	+	+	+	+	+	+	+	+	Sulusoglu (2011b)
Sakarya	-	+	+	+	+	-	-	-	-	Beyhan (2010)
Rize	+	+	+	+	+	+	-	+	+	İslam and Vardal (2009)
Samsun	+	+	+	+	+	+	+	+	+	Macit (2008)
Samsun	-	+	+	-	+	+	+	-	-	Akbulut et al. (2007)
Trabzon	+	+	+	+	+	+	-	+	+	Bostan and İslam (2003)
Trabzon	-	+	+	-	-	-	+	-	-	İslam and Odabaş (1996)

antioxidant (Alasalvar et al. 2005; Kolayli et al. 2003; Halilova and Ercişli 2010).

Much interest has been developed in the last ten years on the antioxidant content of cherry laurel (Halilova and Ercişli 2010; Kasım et al. 2011). Fresh fruit samples of cherry laurel were examined for their total anthocyanin content by using a spectrophotometric differential pH method and cherry laurel genotypes showed differences in their total anthocyanins levels (Kasım et al. 2011). Yaylacı-Karahalil and Şahin (2011), investigated different phenolic constituents and total antioxidant properties of cherry laurel in Trabzon. Phenolic constituents were measured by reverse phase-high performance liquid chromatography (RP-HPLC). Among the chemical characteristics, soluble solid contents and titratable acidity as a malic acid, were used as criteria for selection of the superior types (Table 1).

The nutritional properties of selected types are important for the breeding studies. Gas chromatography-mass spectrometry studies (Ayaz 1997) and HPLC method (Alasalvar et al. 2005) were used to determine the sugar content of the cherry laurel and six sugar forms were identified in fruits; these included monosaccharides (xylose, arabinose, fructose and glucose), trace amounts of disaccharide sucrose, and sorbitol and mannitol as sugar alcohols (Ayaz 1997; Alasalvar et al. 2005). Cherry laurel fruits contain high amount of fatty acids (Ayaz et al. 1997; Halilova and Ercişli 2010). Mineral composition content of cherry laurel fruits was investigated by Kalyoncu et al. (2013) and demonstrated that cherry laurel fruit was rich in potassium.

Studies to serve the breeding of cherry laurel

In general, cherry laurel is considered resistant to disease and is recognized as a species can be grown easily (Sulusoglu and Cavusoglu 2011a). Quaglia et al (2013) reported a new pathogen for cherry laurel that is named “*Diplodia seriata*” found in Italy, and they suggested that this pathogen is potentially dangerous for cherry laurel. Although usage of the cherry laurel is common in Turkey, there have been limited studies including some of cherry laurel demonstration orchards planted in Kocaeli University Arslanbey Vocational School, Black-Sea Agricultural Research Institution, and Atatürk Horticultural Central Research Institution to investigate the cultural practice and performance of cherry laurel.

The second step of the breeding studies

Ex-vitro propagation of cherry laurel plants

Obtaining seedlings of fruit tree species in traditional breeding programs depends on stratification of seeds at low temperatures for several

months and germinating the seed the following spring. Information on germination of cherry laurel seed was limited to germination stage (Young and Young 1992; Norman 1993). The study of pre-treatments and stratification time on seed germination of cherry laurel seeds indicated that seeds soaked in to hot water 90 days after stratification resulted highest germination percentage while application of GA₃ shortened the stratification time of seeds without endocarp (Sulusoglu and Cavusoglu 2014).

After selection, economical mass propagation of cherry laurel is the second step of the breeding program. Vegetative propagation is important in propagation of valuable material, because the genotype of cultivar and varieties is usually highly heterozygote, and the characteristics which distinguish them are often lost by seed propagation (Hartmann et al. 2002). Cherry laurel rooting depends on concentrations of indole-3-butyric acid (IBA) talc or solution application, rooting media, genotype and cutting collection seasons (Riberio et al. 2010; Yazici et al. 2009; Posta 2009; Sulusoglu and Cavusoglu 2009). Semi-hardwood cuttings of sixteen cherry laurel types, selected following pomological studies (Sulusoglu, 2011b) rooted successfully by applying IBA and hence superior rooting genotypes were identified for economic commercial orchard (Sulusoglu and Cavusoglu 2010).

In Vitro Studies

Successful results of micropropagation are very important for the future of breeding studies. The micropropagation approach could be used for genetic transformation, for self-rooted or grafted viral free material production in nurseries that *in vitro* based meristem culture/heat therapy protocol was effective for virus elimination (Kalinina and Brown 2007). *In vitro* proliferation, which can be carried out throughout the year, could provide an alternative, rapid means to produce more clonal material in a shorter period. Initiation cultures were prepared in Murashige and Skoog (MS) medium supplemented with different concentrations of BAP and NAA (Ponchia, 1991) and the maximum shoot proliferation was achieved with 0.5 mg/l BAP and 0.01 mg/l NAA hormone combination. Rooting of micro shoots was better when supplied with naphthalene acetic acid (NAA) than IBA in GA₃ including rooting medium.

Kalinina and Brown (2007) used axillary shoot meristem of cherry laurel to establish *in vitro* cultures. The best Meristem proliferation media was MS consisted of MES (2-(N-Morpholino)-ethanesulphonic acid) and supplemented with 0.5 mg/l BA+0,5 mg/l IBA+2,0 mg/l GA₃. Rooting of micro shoot were achieved in two stage; firstly rooting was

inducted in media including IBA for 4 days and then transferred to IBA-free media for next elongation period. Micro plantlets showed high survival rate in soil. In another study, micropropagation of cherry laurel was achieved with shoot-tip culture (Sulusoglu and Cavusoglu 2013a) in which 2.0 mg/l BAP gave the maximum shoot induction with 4.0 explant in the establishment culture and shoot elongation was better too. Shoot proliferation rate and length of shoots increased when IBA used together with BAP that provided extensive shoot growth and resulted in better rooting in the next step again with IBA. This micropropagation protocol can be used for maintenance of clonal propagation of cherry laurel.

One biotechnological technique that has been beneficial is embryo culture. Embryo culture is a valuable *in vitro* technique for breeding. It is most often used to rescue embryos from interspecific and intergeneric crosses for the material that has importance for breeders. The method also can be used to rescue seedless triploid embryos, produce haploids, overcome seed dormancy, or determine seed viability; useful for understanding embryo morphogenesis and precocious germination (Bridgen 1994). The culture of mature embryos from ripened seeds is used to eliminate seed germination inhibitors or to shorten the breeding cycle, to understand the physical and nutritional requirements for embryonic development (Hu and Wang 1986; Dunwell 1986). Embryo culture can shorten the breeding cycle by overcoming dormancy in seeds. Seed dormancy factors may be localized in the seed coat, the endosperm, or both. Isolating and culturing the embryos in aseptic conditions allows germination and fast growth of embryo that in turn shortens the breeding cycle. Isolated embryos can also be vernalized and this reduces the generation time (Sharma and Gill 1983).

So far the studies have been conducted on embryo culture of cherry laurel are scanty. In one study, mature embryos of cherry and black types of cherry laurel were cultured on MS media supplemented with BAP and IBA hormone combinations. Shoot formation was not observed in cherry type embryos while black type embryos produced seedlings with rudimentary roots which did not show any growth even after transfer to the fresh media. Cold stratification played an important role on the growth of embryos in the same hormone combinations in MS media. Embryo germination and seedlings growth both increased in embryos stratified for 60 days while embryos stratified for 30 days required high hormone concentration to continue to next growth and this protocol would be useful in future breeding program of cherry laurel. Embryo culture of cherry laurel prevented loss of

material during germination stage and shortened the time to obtain seedlings in the same season the seed is collected (Sulusoglu 2012).

Molecular studies

The taxonomic classification within the genus *Prunus* which is mainly based on fruit morphology has been controversial. The revised classification by Rehder (1940), which describes five subgenera; *Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus* and *Prunus* to accommodate variation within the genus, is widely accepted taxonomic group. The subgenera *Padus* and *Laurocerasus* in which cherry laurel is placed in the group is more isolated within the genus *Prunus* (Aradhya et al. 2004). Knowledge of the genetic diversity and relationships among the cultivated species of *Prunus* is important in recognizing the gene pools. Traditional taxonomic classifications provide rough guidelines to species relationships, but molecular evaluations provide detail information about the genetic structure and differentiation within and among taxa useful for plant breeders and geneticist.

Conservation of plant material and truly selection of superior genotypes before cultivation are highly important when used in breeding and gene transfer. If the selected superior types show genetic similarity due to climatic and environmental differences, then repetition will be avoided at the beginning of the breeding studies.

Optimization of SSR fingerprinting technique for species and type level is very important. The first genetic fingerprinting analysis among the 40 cherry laurel genotypes distributed in 6 location of the East Marmara Region of Turkey were reported by Hajyzadeh et al (2013). In this study, genetic similarities and differences in naturally growing 40 cherry laurel genotypes, two sweet cherry cultivars and one laurel type were determined. A total of 12 SSR primers developed from peach, sweet and sour cherry, plum or apricot were used. The eight primer pairs were found to be polymorphic among the cherry laurel genotypes. Cluster analysis showed that assessed genotypes were divided into two major group and several sup-groups while the resolution within the groups remained unsolved.

Before SSR technique, different researchers studied the genetics of cherry laurel using RAPD technique (Sandalli et al, 2005; Lee and Wen, 2001; Bortiri et al, 2001; Turkoglu et al, 2010). Microsatellites have been extensively used in *Prunus* genetics investigations for germplasm characterization (Lacis et al. 2009), determination of genetic diversity (Wünsch 2009), germplasm

management (Cheng and Huang 2009), cultivar identification (Xuan et al. 2009), and mapping genetic linkage (Lalli et al. 2008) in the last decade. If a microsatellites determined in a one species of *Prunus* could be used in the other species that has a transferability and ability to detect polymorphism (Wünsch 2009). Turkoglu et al. (2010) analyzed 10 SSR loci, previously identified for *Prunus* to examine genetic relationship among 23 rootstock candidates belong to different *Prunus* species including five genotypes of *P. laurocerasus* which were grouped in the second sub-cluster of first cluster together with *P. avium* and *P. cerasus* rootstocks. Unweighted pair-group method of arithmetic mean analysis demonstrated that *P. laurocerasus* genotypes had less genetic variation.

A sterile form of common cherry laurel would be useful in curbing its escape from cultivation. Contreras and Meneghelli (2013) attempted to induce polyploidy using *in vitro* exposure of "Otto Luyken" shoots to oryzalin with the objective of developing sterile form of cherry laurel. Shoots were treated for 1, 2, 14, or 28 days with 0, 6.25, 12.5, 25, 50, 100, or 150 μ M oryzalin. Ploidy level of surviving shoots was determined using flow cytometer analysis of DAPI stained nuclei. As expected, when each meristem was analyzed individually, there was a reduced number of mixoploids, as more of the separated meristems were 22x and 44x. Here the target of developing sterile forms should be clearly discussed using those polyploids.

Biological studies

In the fruit trees pollen quality consists of viability, morphological homogeneity, pollen germination and pollen tube growth rate which are very important

component of fertilization and fruit setting; therefore, study of pollen traits is one of the most important approaches for growers and breeders. *P. laurocerasus* L. is specie that needs a pollinator for fruit setting and pollination is very important for quality of fruits (unpublished data, Sulusoglu and Cavusoglu). The study was carried out to determine *in vitro* pollen viability and pollen germination of cherry laurel and the result showed that the viability was changed according to types and tests used. IKI test gave more clear and well-pointed results considerable. Fifteen percent sucrose gave the best pollen germination rate for most of the types (Sulusoglu and Cavusoglu 2013b).

Conclusion

Cherry laurel is a promising fruit for the future and breeding step is going well to selection of superior types and to improve the genotypes for production quality. The physical and chemical characters of cherry laurel types have underlined an interesting variability. Molecular studies could support truly selection of superior genotypes before cultivation and conservation of plant material. Selected types will serve as source of new cultivars of cherry laurel and propagation studies will enable to production of saplings for the new plantation areas. Biological studies will assist to make orchard design, selection and placement of the pollinator but studies at an early stage and needs to be improved. The particular interest given to this Turkey's natural fruit will improve the production diversification and food security, as well as sustainable crop production. As a result of the breeding studies, an alternative fruit crop will be obtained for the growers and the abundant high-quality curative fruit will be supported to the customers.

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