Review Article

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Ekin
Journal of Crop Breeding and Genetics

(2015) 1-1:36-41

Biotechnological approaches in strawberry tree (*Arbutus unedo* L.) breeding

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Citation:
Cavusoglu A, Sulusoglu M and Erkal S 2015. Biotechnological approaches in strawberry tree (*Arbutus unedo* L.) breeding. Ekin J Crop Breed and Gen 1-1:36-41.

Received: 07.06.2014 ***Accepted: 27.10.2014*** ***Published Online: 15.01.2015*** ***Printed: 25.01.2015***

ABSTRACT

Arbutus unedo L. (Strawberry tree) belongs to *Ericaceae* family, is an evergreen shrub or tree, mostly known around Mediterranean region in natural habitat, has valuable medicinal and aromatic properties. The plant is mainly used for its edible fruits. In addition, *A. unedo* have increasing importance in afforestration programmes, beekeeping facilities, ornamental purposes and elucidating plant physiology. Therefore, the species is seemingly a promising fruit plant. Orchards are very limited but increasing demand to the plant will result in initiations of establishment new fruit orchards with superior genotypes viz. tolerant to the abiotic stress, resistant to pests and diseases, rich contents in terms of valuable compounds, convenience to postharvest operations, being visually preferable and suitable to target climate. Plant breeding is an important component to overcome elimination of unwanted features and to reach admirable characters. The main steps in breeding are effective selection, succesfully adaptation and inheritance via transferring the features to subsequent generations. Studies on *A. unedo* breeding via biotechnological approaches were reviewed. In *A. unedo* breeding, only few biotechnological techniques have used in all known for the purposes especially for selection and propagation of the determined best type. The mostly used biotechniques for *A. unedo* were consantrated on *in vitro* propagation and fingerprint analyses to determine genetic diversity and the favourite genotypes wherein the population with compared with pomological features for help in breeding programme.

Keywords: *arbutus unedo*, strawberry tree, breeding, biotechnology

Introduction

Breeding better cultivar has become a highly efficient way to improve plant production for yield, quality and reduced input (Andersen, 2011). The major objective of plant breeding programs is to develop new genotypes that are genetically superior to those currently available for a specific target environment or a target populaton of environments (Wang, 2011). In the first step, conventional plant breeding depends on phenotypic selection. For the purpose, plant breeders deal with certain selection methods. After the process, in the second step; fertilization, hybridization, measurements of inheritance, chromosomal threads, mutation, cell and tissue culture methods, genetic sources, develop new varieties, plant propagation, viz. methods are used to reach the artificial trade varieties. Biotechnological methods sometimes used to either ensure progress of breeding or continuity of the breeding programme. Most of the breeding studies have carried out in the field crop plants. Especially studies on cereals (Korkut et al, 2001; Akar et al, 2008), legumes (Altınbaş, 2003; Toker et al, 2012; Güllüoğlu et al, 2010), oilseed plants (Kaya et al, 2004; Uzun et al, 2003), fiber plants (Karademir et al, 2009), and sugar-starch plants (Barzen et al, 1992) share in the first place. Studies about breeding of horticultural plants (Bolat and Güleryüz, 1992; Pırlak et al, 1997), ornamental and forestry plants (van Eijk and Leegwater, 1975; Hubert and Lee, 2005) and medicinal-aromatic plants (Arslan et al, 2002; Kumar and Gupta, 2008) always have come from behind although everyday we have used them as food, beverages, medicine, environment and ornamenal purposes.

Different geographical regions of Turkey allow fruit to be grown in almost every part of the country, and Turkey has become one of the main fruit production centers of the world (Küden and Küden, 2008). Because of being gene center of apricot, hazelnut, pistachios, fig and grape, the first studies have focalized on the plants in Turkey (Balkaya and Yanmaz, 2001). Scientific studies on fruit breeding (Bolat and Güleryüz, 1992; Pırlak et al, 1997; Uzun et al, 2008; Acar et al, 2013; Aksu, 2013; Ozdemir et al, 2013), especially on new promising fruit species have performed for a while.

Arbutus unedo, is one of the new promising fruits species, that is newly introduced and studies. The strawberry tree is an evergreen shrub or small tree (Figure 1a). In autumn, the white or pinkish flower clusters, along with the 20-25 mm orange fruits from the previous year (Figure 1b), contrast to the dark foliage (Gratani and Ghia, 2002). The plant natively takes place in Northern Africa (Algeria, Morocco,Tunisia); in Western Asia (Turkey, Lebanon, Syria, Cyprus); in Northern Europe (Ireland); in East Europe (Ukraine); in Southeastern Europe (Albania, Bulgaria, Croatia, Greece, Italy) and in Southwestern Europe (France, Portugal, Spain) (USDA-ARS-GRIN, 2013). According to our personal observation, the species is well-adapted in Turkey.

Although the main usage of the plant is fruit consumption, the fruits or leaves have valuable food content and medicinal and aromatic properties (Ayaz et al,2000; Pawlowska et al, 2006; Males et al, 2006; Fortalezas et al, 2010; Ruiz-Rodriguez et al, 2011; Özcan and Hacıseferoğulları, 2007; Pabuçcuoğlu et al, 2003). The plant can be used for recovery after forest damage or burning via lignotuber (Canadell and Lopez-Soria, 1998; Konstantinidis et al, 2006) for ornamental purpose (Metaxas et al, 2008) via leafy branch in cut-flowers or via habitus and flowers in plantation in gardens and for beekeping facilities (Dalla Serra et al, 1999; Rasmont et al, 2005). At the same time the evergreen *A. unedo* was subjected to plant physiology studies (Panicucci et al, 1998; Navarro et al, 2007) to reach conclusions on photosynthetic response or water usage under different treatments.

Firstly, on the way of conventional plant breeding, *A. unedo* selections were performed depending on pomology, phenology and morphological features of selected different plants or selected different populations (Karadeniz et al, 2003; Şeker et al, 2004; Yarılgaç and İslam, 2007; Celikel et al, 2008; Sulusoglu et al, 2011; Molina et al, 2011). Unfortunately other conventional plant breeding methods could not be reached in this plant.

The purpose of this review was to put out the biotechnological steps conducted to help *A. unedo* breeding that may be progressed.

In vitro tissue culture techniques

The techniques include studies on *in vitro* fertilization, embryo culture and protoplast fusion containing wide hybridization method, haploids method, somaclonal variation method and studies on propagation of plants, synthetic seed, pathogen eradication and germplasm preservation containing micropropagation topic (Brown and Thorpe, 1995). For this purpose very few studies have been reached about A. unedo.

One of the firsts was about *in vitro* breaking of dormancy of axillary buds (Rodrigues et al, 2001). The researchers used nodal segments from adult plant in November and February considered for experiment, corresponding to bud dormancy and breaking bud dormancy period respectively. At the same time they studied the effect of cutting method (surgical blade or laser cut). For the study, determined medium was used containing zeatin and dithiothreitol under certain time light/dark photoperiod. It was reported that, the laser cut method considered for overcoming problems with *in vitro* micropropagation of *A. unedo* in winter which enables higher productivity through the year.

In another study, micropropagation of *A. unedo* was achieved (Mereti et al, 2002) and the plantlets were succesfully acclimatized. For this purpose, nodal segments from defoliated actively growing shoots were cultured in medium containing benzyladenine for shoot culture and indole-3-butiric acid or indole-3-acetic acid for root culture under photoperiod. In the rooting experiment medium with or without peat:perlite (1:4 v:v) mixture to addition at a 1:1 (v:v) ratio were also tested. Apart from the mentioned study, a very similar study on *in vitro* rooting was published in 2003 (Mereti et al, 2003).

On the other hand Canhoto et al. (2007) obtained somatic embryos in all stages from *A. unedo* leaf explants cultured in a modified medium with benzyladenine and naphthalene acetic acid.

Gomes and Canhoto (2009) studied on micropropagation of six selected adult strawberry trees. The study was started with shoot apices and nodal segment under dark condition for a week, and after then photoperiod condition. Different compounds added into the main medium for plant formation were used in the study. Several factors of the experiment were detected. One of the important result was observed is that shoot multiplication might be influenced by the genotypes. Some clones were found difficult to propagate and this indicated being related with the levels of endogenous growth regulators in plant genotypes.

Gomes et al, (2009) studied on micropropagation of selected *A. unedo* trees and somatic embryogenesis. The importance and difference of the study was achievement of somatic embryo conversion.

According to another study (Gomes et al, 2010), effect of different combinations of benzyladenine or other cytokinins or naphthalene acetic acid with benzyladenine interaction with genotypes were subjected. The results indicated that multiplication rate depends on the *A. unedo* genotypes and plant growth regulators types.

Gomes (2011) performed another *in vitro* study on testing mycorrhizae inoculation that can improve plant adaptation and tolerance to stressful conditions. Results and implications of the study on *A. unedo* breeding program were discussed.

El-Sayed El-Mahrouk et al. (2010) aimed at their study to develop an efficient method for propagation of *A. unedo* through adventitious shoots and somatic embryogenesis using segments of *in vitro* obtained axillary shoots. Axillary shoots induction were induced in plant growth regulator free medium. When thidiazuron added, medium gave the best for shoot multiplication. Embryogenic callus and somatic embryos on the calli were obtained from internodal segments at relatively higher concentration of benzyladenine and naphthalene acetic acid combination. After then plantlets continued to next development in hormone-free media.

Plant genetics and molecular biology techniques

The analysis of the genetic diversity of *A. unedo* takes the first place among the researches in this topic. To evaluate genetic diversity or genetic similarity -in association with phenology, morphology, physiology, geograpy and/or ecology-, molecular markers are useful instruments in plant improvement and breeding via determination certain genotypes.

Genetic diversity among the nine Tunusian *A. unedo* populations were assessed using sixty five polymorphic RAPD loci (Takrouni and Boussaid, 2010). According the results of the study, three groups of the populations analysed and the groups were not related with geographical or bioclimatical origin.

In another genetic variability study, DNA extraction from leaves of *A. unedo* was optimised first (Sa et al, 2011) because concentration, purity and quality of the extracted DNA is important. After the procedure, the DNA samples were used in molecular analysis based on both RAPD and ISSR to indicate diversity (Lopes et al, 2012). Results proved that there were high levels of gene flow among populations so this caused low differentiation. The cluster analysis showed that only one population formed a distinctive cluster, remaining formed a second cluster due to their geographical approach.

Gomes et al. (2013) have analysed the genetic relationship among twenty seven *A. unedo* genotypes from a broad geographic range using twenty RAPD and eleven SSR markers. Results indicated that no correlation was found between the markers or geographical origin.

As distinct from the above experiments, Zamboni et al. (2008) extracted the total RNA from *Arbutus unedo* and several other woody-plants for gene expression analysis as represented non-model species for molecular biology. Expression of the agricultural important gene will lead to the progress in *Arbutus unedo* breeding.

Conclusion

On behalf of biotechnology, significant progress has been made in plant breeding using cell or tissue culture *in vitro* and studies or manupulation in genetics and molecular techniques of plants, in particular in crop plants. *A. unedo* is a newly promising horticultural species so few studies have conducted on the way of its breeding. For the present, conventional selection with the help of studies on genetic similarities, and micropropagation of the choosen superior types came to the fore. In addition to these before studies in breeding of A. unedo, next studies can be expected to be subject and/or progress about haploids in crosses, fertilization, changes in ploidy, screening of diseases, stress etc. conditions, rootstock breeding, breaking seed or bud dormancy, obtaining variation via cell, meristem, anther, callus and protoplast cultures, somatic and zygotic embryogenesis and cryopreservation etc. in *in vitro*. At the same time breeding in *A. unedo* can cause development new cultivar in pest, disease, weed, pesticides-resistance, bringing in physiological behavior as photosynthesis, nutritional storage, nitrogen fixation, postharvest resistance positively and gene silecing etc. topics in genetics and molecular biology as achieved previously in certain plants. This point should be emphasized again that studies on *A. unedo* breeding (conventional or high-tech applications) are in the spring of its study life when compared with numerous field and horticultural plants

Figure 1. (a) *Arbutus unedo* L. in natural habitat, (b) One of the *Arbutus unedo* L. genotype studied by Sulusoglu et al, (2011) selection studies.

Acknowledgements

The study was presented at International Plant Breeding Congress,10-14 November 2013 Antalya/ Turkey, and published as an abstract in The Congress Abstract Book p.526.

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