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Aysun Cavusoglu
Kocaeli University, Faculty of Agriculture and Natural Sciences, Department of Plant, Protection, TR41285 Kocaeli, Republic of Turkey

Dogu Can Anil Aygun
Kocaeli University, Graduate School of Natural and Applied Sciences, Department of Horticulture, TR41001 Kocaeli, Republic of Turkey

Corresponding Author:
Aysun Cavusoglu
Kocaeli University, Faculty of Agriculture and Natural Sciences, Department of Plant, Protection, TR41285 Kocaeli, Republic of Turkey

Responses of different substrates and varieties on growth of blueberry (*Vaccinium corymbosum* L.) cuttings

Aysun Cavusoglu and Dogu Can Anil Aygun

Abstract
The one of the most traded berry is blueberry, *Vaccinium corymbosum* L., belongs to Ericaceae family has an increasing interest as agricultural practices and scientific area. Blueberry is grown for the production of fruits. The fruits are considered a health food due to the rich content of minerals, trace elements, and phenolic compounds. The study was carried out in Kocaeli city in Turkey, one of the cities that share Black Sea coast, between October, 2019-January, 2020. In this study, current year shoot cuttings were collected in the study day from mother plants of three varieties ('Duke', 'Brigitta' and 'Bluecrop'). Shoots from branches in 8 cm were prepared by removing all leaves and planted as softwood cuttings in two different substrates (peat and perlite) under glasshouse conditions to observe; viability rate (%), bud sprouting rate (%), rooting rate (%) and callusing rate (%). Results obtained after 4 months indicated that viability rates were 83,3% in 'Duke', 79,17% in 'Brigitta' and 62,50% in 'Bluecrop' that showed statistical differences in peat. In perlite the viability rates were 70,38% in 'Duke', 70,83% in 'Brigitta' and 75% in 'Bluecrop' and showed no differences between varieties. Even the used media did not affected the viability rates in each variety. Bud sprouting rate changed between 13,3%-44,43 in all cuttings and did not show statistical importance among varities or substrates. Only differences in callusing rate (52,2% in 'Duke', 13,07% in 'Brigitta' and 8,9% in 'Bluecrop') were observed only in perlite substrate. It should be emphasized that no rootings observed in used media or varities under our study conditions. According to the data, rooting is an issue that needs to be worked on, especially under nursery condition on the varities.

Keywords: *Vaccinium corymbosum*, cutting, callusing, peat, perlite

Introduction
Vaccinium corymbosum L. is commercially produced plant species for its edible berry fruits. Plantation areas of the woody shrub plants has increased worldwide since the early 2000s. While America continental especially U.S. is the leader in terms of land size and yield of the plants, there has been also a serious interest for the plant from the countries of other continents (DeVetter *et al*., 2015) [1]. The most important reason for this can be the progressive studies on the fruit content and the substances proved to be very important for health. Most of the studies focused on their valuable compounds in berries which have high biological activity for example phenolics (Rimando *et al*., 2004; Dragović-Uzelac *et al*., 2010; Gato *et al*., 2020) [2-4], sugar and organic acids (Wang *et al*., 2008; Zorenc *et al*., 2016) [5, 6], anthocyanins and vitamins (Aires *et al*., 2017; Nadulski *et al*., 2019) [7, 8]. It will be more important than before obtaining healthy, known, adaptable sapling for these increased production areas. For this purpose there are some studies conducted for the production of saplings with different methods. For example propagation of the plant via softwood cuttings (El-Shiekh *et al*., 1996; Litwińczuk *et al*., 2005; Celik and Odabas, 2009) [9-11], semi-hardwood cuttings (Braha and Rama, 2018) [12], hardwood cuttings (Akbulut *et al*., 2015; An *et al*., 2019; Braha *et al*., 2020) [13-15], microcuttings (Pacholczak and Nowakowska, 2015) [16], and micropropagation (Rowland and Ogden, 1992; Ostrolucká *et al*., 2004; Vescan *et al*., 2012) [17-19], have been woked on. In most of the articles, different cultivars, substrates, growth environment and some chemicals that caused rooting have studied by researchers. The adventitious roots formation in cuttings from vegetative tissue depends on a series of exogenous and endogenous factors (Druege *et al*., 2019) [20]. Actually when organic sapling aimed, usage of the chemicals and usage of all media or substrates are not possible, but it is possible to choose plants that are more prone to rooting.

The study aimed plant growth responses of cuttings on the road of rooting under the most unfavorable growing condition to choose true cultivar and true substrate for further studies especially in organic plantation aims.

Materials and Methods

The study was carried out in Kocaeli city in Turkey, between October, 2019-January, 2020. In this study, current year shoot cuttings were collected in the study day from mother plants of three varieties ('Duke', 'Brigitta' and 'Bluecrop') that purchased from a commercial nursery garden. The ten mother plants for each varieties were grown outdoors in large pots separetely (Fig. 1).Healthy and uniform shoots from branches in 8 cm were prepared by removing all leaves and planted as softwood cuttings (Fig. 2) in two different substrates (peat and perlite) under unheated greenhouse conditions on the 12th October of 2019. Each cutting placed in separete black plastic pot which were filled with 0.2 dm^3 peat or perlite (Fig. 3). Viability rate (%), bud sprouting rate (%), rooting rate (%) and callusing rate (%) of the cuttings observed and calculated after 4 months of first placed (Fig. 4). While the viability rate was calculated from all cuttings, bud sprouting, rooting and callusing rates were calculated from viable cuttings in the study. The experiment laid out in Completely Randomized Design (CRD), consisted of 2x3 treatments with two substrates, three varieties and three replicate for each treatment. Each sub-plot consisted with eight, an application consisted with twenty four, all study consisted with one hundred forty four cuttings in total. The recorded data were analysed through statistical software for observation the parameter were statically significant or not at 0.05% level.

Fig 1: *Vaccinium corymbosum* L. (a) The initial plants of three cultivar, (b) Examples of each cultivars 'Duke', 'Brigitta' and 'Bluecrop' from left to right.

(a) (b) (c)

Fig 2: Prepared softwood cuttings of *Vaccinium corymbosum* L. belong to the three cultivars just before planted to the substrates (a)'Duke', (b)'Brigitta', (c)'Bluecrop'.

Fig 3: *Vaccinium corymbosum* L. softwood cuttings belong to the three cultivars after planted to the substrates just before being distributed according to completely randomized design.

(a) (b) (c)

Fig 4: *Vaccinium corymbosum* L. softwood cuttings 4 months after planted (a) In peat, (b) In pelite, (c) Callusing in perlite under glasshouse condition.

Results and Discussion

After four months of cuttings growth in the pots under unheated glasshouse condition on the 12th January of 2020; softwood cuttings of the three highbush blueberry varieties exhibited more viability in peat in "Duke" at a ratio of 83.3% (Table 1, Figure 5a). In the same parameter; peat substrate has the lowest result in "Bluecrop" with 62.50%. Perlite showed more stability on cutting viability rate and changed between 70.38-75% in varieties and showed no significant differences. Bud sprouting is also an important factor for marketable sapling and changed between 13.3-21.39% in peat and between 35.68-44.43% in perlite in varieties. In this manner perlite was found succesful but there is no statistical differences (Table 2, Figure 5b). Unfortunatelly there are no rooting in the used varieties and the used substrates in our study condition (Table 3, Figure 6a) but callusing was found higher in perlite than peat in which no callusing was observed. Callusing also showed the highest rate in "Duke" with 52.2% in perlite (Table 4, Figure 6b). In one of the study (Finn *et al*., 2003) [21], on genotype x environment interactions in 20 highbush blueberry at two locations, it has been found that survival rate changed between 46.1-100% in genotypes and between 56-92.4% in locations in the USA. Similarly Çelik (2009) [22], has studied on performance of 5 blueberry varieties and found that all measured parameters include vegetative and berry characteristics highly depends on varieties in Eastern part of Turkey. According to an *in vitro* propagation study (Ostroluckά *et al*., 2004) [18] in Slovakia, with 5 blueberry cultivar include "Duke" and "Bluecrop", it was emphasized that multiplication depends on not only used plant growth regulators but also depends on species and cultivars. In the study "Duke" is also gave the highest responce in all used blueberry cultivars. Pacholczak and Nowakowska (2015) [16], have studied on rhizogenesis and related topics on *Vaccinium corymbosum* 'Bluecrop' and 'Duke' with IBA, commercial rooting powder and salicylic acid on the *in vivo* with *in vitro* derived tip of microcuttigs in Poland on February. They used peat and perlite mixture as basal subsrate. Their results showed that in both cultivars gave the lowest results in control among all applications. Akbulut et al. (2015) [13], also studied on rooting of 10 cultivars of *Vaccinium corymbosum* in Turkey. They used woody cuttings and different plant growth regulators in different concentrations. In the study, importance of cultivar differeces and using plant growth regulators at appropriate dose were found. Control is also gave the lowest results than medium level of used dose but not than highest dose of used PGRs. In a study (Çelik, 2016) [23], leafy softwood microcuttings from two cultivars of *Vaccinium corymbosum* ('Jersey' and 'Berkeley') were used in 11 substrate for obtain the best rooting medium with the help of a dose of IBA in July under mist in a greenhouse in Turkey. Importance of substrate differences on rooting was strickly emphasized for rooting in this study.

Conclusion

The research results show that, the three varieties ('Duke', 'Brigitta' and 'Bluecrop') are not rooting easily with softwood cuttings in peat or perlite in October planting time without any plant growth regulator, bed heating and mist application in our study conditions. Their viability rate can changed in peat, additionally it was found between 62.50-83.30% in all experiment. Bud sprouting rate was higher in perlite than peat but not showed statistical differences and this results found between 13.3-44.43% in all experiments. No callusing is observed in peat while perlite gave callusing in all cultivars and it was showed statistically difference between substrates in 'Duke'. Even cultivars showed differences in callusing in perlite. The parameter changed between 0-52.2% in all treatments. Unfortunately there was no rooting. When we search the literature not only family or genus but also cultivar differeces can be responsible on rooting, in addition cutting type, time of year, substrate, propogation methods and exogenous plant growth regulators strickly effect on rooting of cuttings. All this issues must be considered in the studies to be carried out afer that for organic or conventional agricultural aims.

Table 1: Viability rate in cuttings of blueberry (*Vaccinium corymbosum* L.) varieties in different substrates after 4 months

Varieties	Viability (%)		
	Peat**	Perlite**	Average in Variety
Duke	83,30 a*	70,38	76,84
Brigitta	79,17 ab	70,83	75,00
Bluecrop	62,50 b	75,00	68,75
Average in Substrate	74,99	72,07	73,53

*Different small letters indicate variety differences only in peat at $P \leq 0.05$ level, **There is no differences between substrates in each variety

Table 2: Bud sprouting rate in cuttings of blueberry (*Vaccinium corymbosum* L.) varieties in different substrate after 4 months

Varieties	Bud Sprouting (%)		
	Peat*,**	Perlite*,**	Average in Variety
Duke	13,3	44,43	28,87
Brigitta	21,39	35,68	28,54
Bluecrop	16,63	39,28	27,96
Average in Substrate	17,11	39,80	28,46

*There is no differences between substrate,
** There is no differences between varieties for each substrate

Table 3: Rooting rate in cuttings of blueberry (*Vaccinium corymbosum* L.) varieties in different substrate after 4 months

Varieties	Rooting (%)*		
	Peat	Perlite	Average in Variety
Duke	0	0	0
Brigitta	0	0	0
Bluecrop	0	0	0
Average in Substrate	0	0	0

*There is no rooting

Table 4: Callusing rate in cuttings of blueberry (*Vaccinium corymbosum* L.) varieties in different substrate after 4 months

Varieties	Callusing (%)		
	Peat	Perlite	Average in Variety
Duke	0 B*	52,20 a**A*	26,10
Brigitta	0 B	13,07 b B	6,54
Bluecrop	0 B	8,90 b B	4,45
Average in Substrate	0	24,72	12,36

*Capital letter indicates substrate difference in same variety at $P \leq 0.05$ level, **Small letter indicates variety differences in only perlite substrate at $P \leq 0.05$ level.

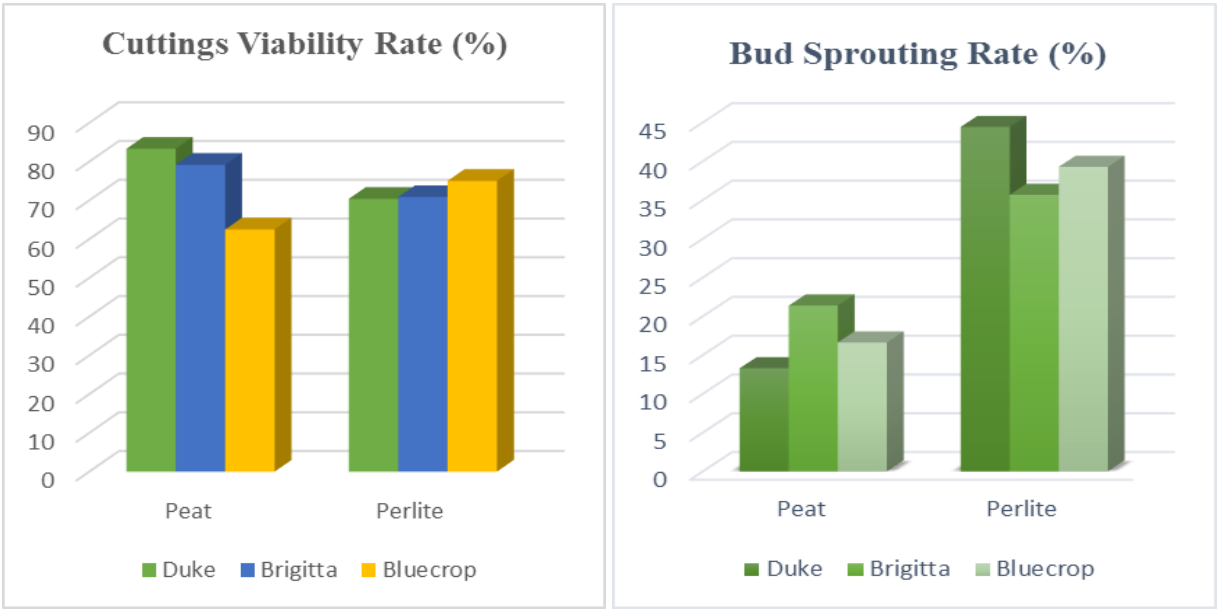

Fig 5: *Vaccinium corymbosum* L. cultivars in peat and perlite (a) Cuttings viability rate (%), (b) Bud sprouting rate (%)

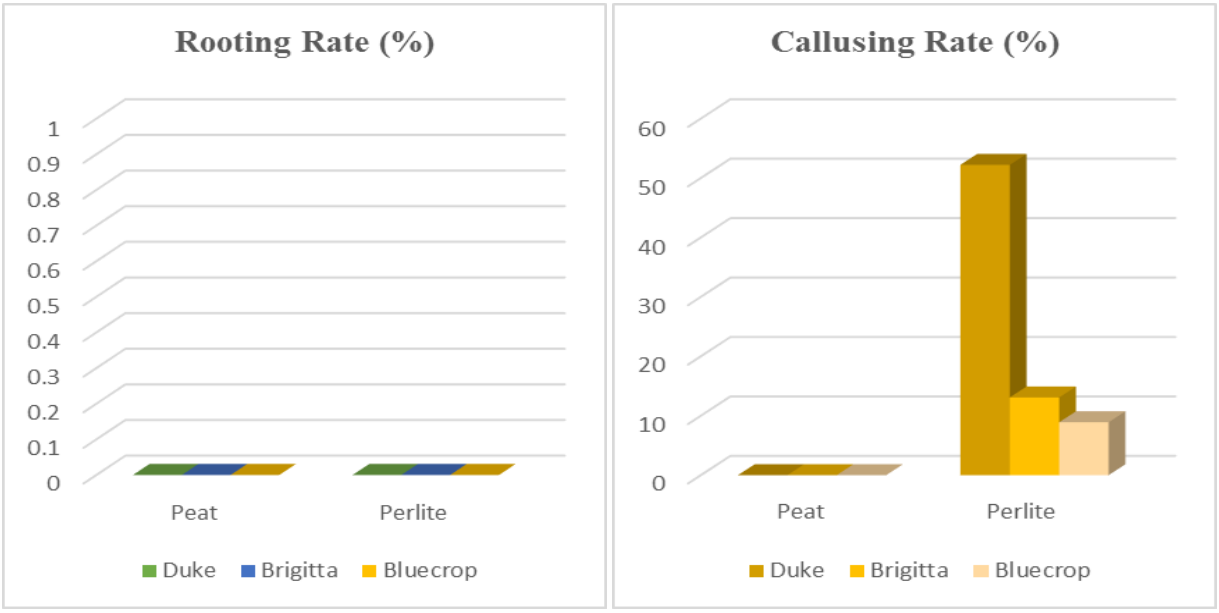

Fig 6: *Vaccinium corymbosum* L. cultivars in peat and perlite (a) Rooting rate (%), (b) Callusing rate (%)

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