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Vegetative Habitus and Fruit Production of Self-rooted Cherry Cultivar ‘Hedelfingen’ Wild Type and Somaclonal Grafted on ‘Gisela 6’ and ‘Colt’ Rootstock

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Abstract: The study considers the morphological and physiological behaviour of self-rooted sweet cherry CV (Cultivar) ‘Hedelfinger’ wild type (H) and somaclonal (HS) grafted on ‘Gisela 6’ and ‘Colt’ rootstock. The somaclonal showed reduced vegetative vigour without any variation of the natural tree’s architecture. The rootstock ‘Gisela 6’ caused change in genotype habitus inducing a spreading shape, while ‘Colt’ increased trunk diameter and height. Fruit quality and size were not affected by genotype nor rootstock. ‘Gisela 6’, from these preliminary data, had proved the most suitable rootstock for both genotypes since it reduced the tree size and vigor and induced early bearing and the production of a greater number of fruiting spurs.

Key words: Cherry CV ‘Hedelfingen’, growth habit, self-rooted somaclonal, ‘Gisela 6’, ‘Colt’, rootstock.

1. Introduction

Genetic variability occurring in plant tissue culture may have novel agronomic traits that can not be achieved by conventional breeding. Effect of root type and clone of ‘Schattennmorelle’ sour cherry upon growth and productivity was studied [1]. A lower vigor was significantly manifested by reduced increase of trunk, compared with the tree rootstocks. Self-rooted trees gave a lower yield per tree; however, their cropping efficiency index was 23-28% higher than that of trees grown on seedling rootstocks. Tissue culture generates a wide range of genetic variation in plant species, which can be incorporated in plant breeding programmers [1]. By *in vitro* selection, mutants with useful agronomic traits e.g. salt or drought tolerance or disease resistance, can be isolated in a short duration [2]. Moreover, root genotype affects many physiological parameters as water relations, leaf gas exchange and chlorophyll a fluorescence pigments, metabolites and fruit quality indices of cherry cultivar growing on rootstock with differing size-controlling potentials [3]. Other important agronomic traits of rootstock include compatibility, good tolerance to root hypoxia, water use efficiency, aptitude to uptake soil nutrients, tolerance to soil or water salinity, resistance/tolerance to pests and diseases, such as nematodes, soil-borne fungi, crown gall, bacterial canker and several virus, viroids and phytoplasms [4].

The lease of somaclonal as commercial cultivars has only sporadically included woody temperate fruit species. Somaclonal variation has provided a new and alternative tool to the breeders for obtaining genetic variability relatively rapidly and without sophisticated technology in horticultural crops, which are either difficult to breed or have narrow genetic base [5]. Somaclonal variant, named HS, regenerated from *Prunus avium* ‘Hedelfingen’ leaf tissue, exhibited different pattern of growth and development and reduced vigor respect to wild type under different light qualities of *in vitro* and early ex vitro conditions [6, 7]. The HS, somaclonal fruits quality at early unripe (green) and full ripe (dark red) stages did not show main biochemical differences respect to wild type fruit

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[8]. The proteomic analysis identified 39 proteins differentially accumulated between H and HS fruits at the two ripening stages, embracing enzymes involved in several pathways, such as carbon metabolism, cell wall modification, stress response and secondary metabolism. The evaluation of fruit phenolic composition by mass spectrometry showed that HS sweet cherries have higher levels of procyanidin, flavonol and anthocyanin compounds [9].

Tree size reduction is one of the most relevant aspects of cherry plant cultivated in field. New scion somaclonal HS could also represent a genetic source for breeding programs. The present research pursues the characterization of the somaclonal HS grafted on 'Gisela 6' (G6) and 'Colt' (Ct) in orchard system after 5 years of cultivation.

2. Material and Methods

2.1 Cultivation System

Self-rooted cherry trees CV 'Hedelfingen' (H) and its somaclone HS were transplanted in May 2005 in the "Fondazione Minoprio" Orchard (Vertemate-Como, Italy) and they blossomed in March 2011. Cherry trees CV 'Hedelfingen' and somaclonal HS, grafted on 'Gisela 6' (G6) and 'Colt' (Ct) rootstocks in October 2007 at the Department of Agricultural Science of University of Bologna, were transplanted in February 2008 in the "F. Dotti" Experimental Farm of University of Milan, and they blossomed on 16th April 2010.

2.2 Plant Vigor

Plants surveys were carried out on May/June of the first blossoming year (2010/2011). The data were detected after dividing the canopy into three segments: basal, middle and apical as described in Piagnani, M. C., et al. [6]. The following parameters were measured: collar stem diameter at 20 cm, plant height, long branch of one year, internodes length, leaves (width, length, area and petiole length), insertion angles and extension, n°/buds/fruit and wood in the branches of two and three years.

On self-rooted trees, 20 representative branches from wild type cherry CV 'Hedelfingen' and HS were chosen, while on grafted ones, the measurements were performed on all the branches present on the tree.

2.3 Fruit Quality

Fruit sugar content was assessed by digital refractometer (Atago, Tokyo, Japan) and acidity by titration with NaOH 01 N (Compact-S Titrator; Crison, Modena, Italy) on cherry juice and skin color by Minolta CR300. Reflectance colorimeter (Chromameter CR-300; Minolta, Osaka, Japan) within two ripening classes, measuring a* and b* minimum, maximum and average value of the L*a*b* CIELAB scale, represented the change in color green to red (a* value) and blue to yellow (b* value).

2.4 Statistical Analysis

The data processing was performed by ANOVA (Analysis of Variance) using SPSS.18 software (SPSS Inc., Chicago, IL, USA). Significant differences were calculated by Tukey's mean test.

Differences at $p \leq 0.05$ were considered as significant.

3. Results and Discussion

3.1 Plant Vigor

Self-rooted somaclonal (HS) has shown, respect wild-type (H), a reduced trunk diameter and height and longer shoots and internode, while branch angles do not show any difference (Table 1A). The rootstock has not influenced the HS development except for the increase of shoot length by 'Gisela 6' rootstock. On the contrary, 'Hedelfingen' grafted on 'Colt' has increased trunk diameter and tree height, but has significantly reduced internode length (Table 1B). Tree habitus has not modified in self-rooted genotypes, while 'Gisela 6' rootstock has induced a wider shape than 'Colt' increasing both crotch and extension angles, since the average angle of insertion and extension has been rated 65° and 48° for 'Colt' and 81° and 73° for 'G6' (Table 1B).

Size leaf measures have taken into account: lamina length, lamina width and leaf area; width/length and petiole length. Self-rooted HS has shown shorter leaves and petiole (Table 2A) and the interaction of genotype and rootstock has been manifested by the reduction of leaf width in H/'G6' combination. HS has shown larger leaves in self-rooted plants and in both the rootstock combination. Rootstock 'G6', in general, leads to a more elongated shape of the leaf, while the combination 'H' on 'Ct' gives the less elongated. The combination HS/'G6' gave the longer petiole, while in self-rooted, the somaclonal petiole was shorter than the wild type (Table 2B).

3.2 Fruit Quality

Skin color, determined by CIELAB scale, did not show differences in fruit of the two genotypes within each ripening class (Table 3).

Fruit sugar content (SSC (Soluble Solid Content)) and acidity were not different within the three different ripening classes and the two genotypes (Table 4).

Rootstock 'Gisela 6' induced a significant increase in the number of flowers per inflorescence and this effect was higher in the Somaclone HS instead wild type H (Table 5).

Self-rooted cherry trees were transplanted on May 10th, 2005 after four years on May 8th, 2009. H (wild type) was very vigorous while HS (somaclone) was very small (Fig. 1).

Both the cultivars, WT (Wild Type) and HS grafted on 'Gisela', blossomed at the second year (Fig. 2). Only 25% of the trees grafted on 'Colt' blossomed in the second year and 60% of them did it in the third season.

Cherry fruit at the first ripeness stage were collected on May 25th, 2013 (H = WT; HS = somaclonal) (Fig. 3) and on May 30th, 2013 for the second ripeness stage (Fig. 4).

The average cherry production was respectively 785 g for the 'Wild Type' and 1,222 g for the somaclonal variant; these differences were not statistically significant.

Table 1 Trunk diameter, height, one year shoot and internode length, crotch (α°) and extension (β°) angle. (A) self-rooted (§n = 5) and (B) grafted trees (Xn = 8): genotype and rootstock interaction.

Genotype		Trunk diameter (cm)	Height (cm)	One year shoot (cm)	Internode length (cm)	Crotch (α°)	Extension (β°)
(A)	H	10.8§	341.6§	46.9	3.9	65.3	65.7
	HS	6.2*	243.5*	54.1*	4.4*	67.8	68.8
(B)	H/'G6'	3.0 a	165.4 a	33.3 ab	2.8 b	78.8	71.3
	H/'Ct'	4.5 b	233.7 c	28.5 a	2.3 a	63.7	45.6
	HS/'G6'	3.9 a	183.3 a	36.6 b	2.9 b	83.9	75.9
	HS/'Ct'	3.3 a	181.8 a	27.8 a	2.7 b	67.7	50.5

Note: * and different letters represent significant differences for $P > 0.05$.

Table 2 Leaf measurements: lamina length, lamina width and leaf area, width/length and petiole length. (A) self-rooted and (B) grafted trees: genotype and rootstock interaction.

Genotype		Lamina length (cm)	Lamina width (cm)	Lamina area (cm^2)	Lamina width/length	Petiole length (cm)
(A)	H	14.9	7.3	109.5	0.49	3.2
	HS	14.4*	7.4	107.2	0.52**	2.8*
(B)	H/'G6'	12.3 a	5.3 a	66.0 ab	0.43 a	3.7 a
	H/'Ct'	11.1 a	5.6 b	63.6 a	0.51 c	3.7 a
	HS/'G6'	13.5 a	5.8 b	79.5 c	0.43 a	4.5 b
	HS/'Ct'	12.4 a	5.7 b	71.2 b	0.47 b	3.3 a

Note: * and different letters represent significant differences for $P > 0.05$.

Table 3 Fruit skin color as assessed by Minolta for wild type (H) and somaclonal (HS) within the two ripening classes (a* and b* minimum, maximum and average value of the L*a*b* Minolta CIELAB scale).

CIELAB	Genotype	Date	Ripening class	Number	Min	Max	Average	Standard deviation
a*	H	May 25th	1	125	22.1	44.0	35.0	4.2
a*	H	May 30th	2	124	5.7	30.7	18.0	5.8
a*	HS	May 25th	1	67	19.5	44.3	34.4	5.7
a*	HS	May 30th	2	58	6.1	28.2	16.0	4.8
b*	H	May 25th	1	124	2.5	18.8	11.3	3.0
b*	H	May 30th	2	124	0.9	14.5	3.9	2.6
b*	HS	May 25th	1	67	1.1	18.8	11. 0	4.2
b*	HS	May 30th	2	58	0.9	15.8	3.3	2.6

Table 4 Fruit qualityfor wild type (H) and somaclone (HS) within the two ripening classes (ns = not significant).

Genotype	Date	SSC (Brix°)	Acidity (meq)
H	25/05	20.6 ns	--
	30/05	20.4 ns	11.2 ns
	6/06	16.9 ns	8.5 ns
HS	25/05	21.4 ns	--
	30/05	19.7 ns	11.3 ns
	6/06	17.1 ns	8.3 ns

Table 5 Flowers per inflorescence for wild type (H) and somaclone (HS) grafted on 'Colt' (C) and 'Gisela 6'(G6) rootstock.

Genotype	Rootstock	Inflorescence flowers
H	'Colt'	2.8 a
	'G6'	6.3 b
HS	'Colt'	3.5 a
	'G6'	8.6 c

Note: Different letters represent significant differences for $P > 0.05$.

'Hedelfingen' (a) HS Somaclonal (b)

Fig. 1 Self-rooted trees (H and HS), transplanted on May 10th, 2005, after four years on May 8th, 2009.

Fig. 2 WT and HS trees grafted on ‘G6’ blooming at second growing season.

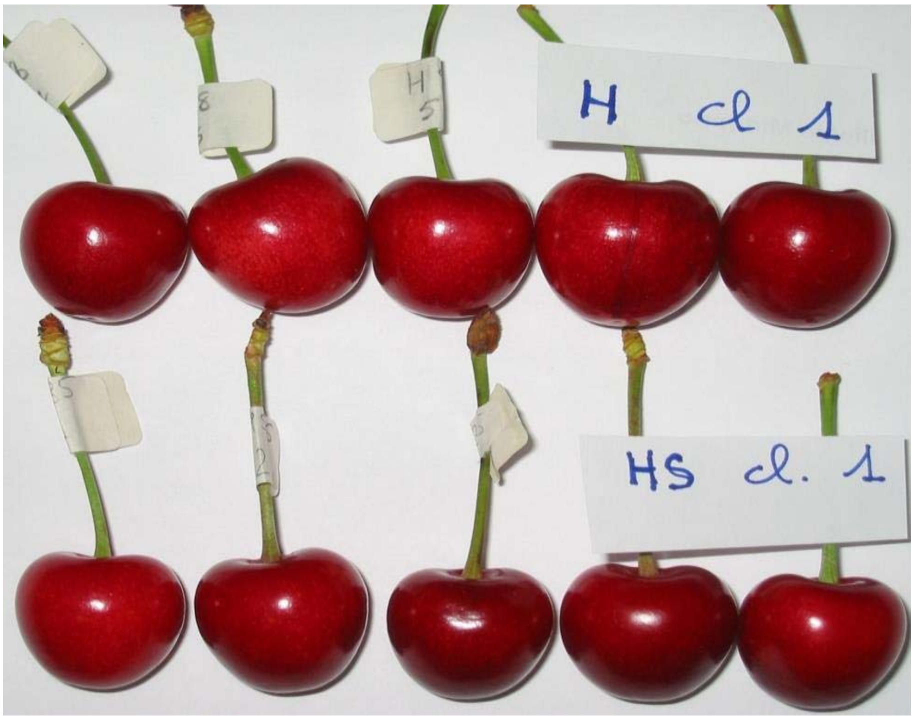

Fig. 3 First ripeness stage of cherry fruit collected on May 25th, 2013 (H = WT; HS = Somaclonal).

Fig. 4 Second ripeness stage of cherry fruit were collected on May 30th, 2013 (H = WT; HS = Somaclonal).

4. Conclusion

The somaclone HS was less vigorous than the wild type genotype, without any influence on natural tree's architecture based on crotch and extension angles, which did not differ between H and HS. HS was less productive but fruit quality did not differ from wild type.

The rootstock 'Gisela 6' have changed growth habit because, irrespective of genotype, that when grafting them has assumed an expanded habitus.

With regard to the vigor, rootstock has had more influence on the 'wild type' than Somaclonal, since 'Colt' induced an increase of trunk diameter and height, and a reduction of the length of the internodes. Two genotypes (WT and HS) were differently influenced by the rootstock in terms of size and shape of the leaves. 'Gisela 6' contributed to a significant increase in leaf surface.

Size and color of drupe are one of the most important quality factors for attaining a high price and in this view, any change due to somaclonal variation has to be carefully considered. Both fruit size, shape, the trend of fruit color change during the ripening process and the main parameters of fruit quality as assessed in the wild type and the Somaclone were the same. Although H and HS fruit final weight were the same, H reached its maximum quicker than HS.

'Gisela 6', from these preliminary data, had proved the most suitable rootstock for both genotypes since it induced early bearing and the production of a greater number of fruiting spurs.

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