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Evaluation of 'Tonda di Giffoni' hazelnut (*Corylus avellana* L.) clones

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ABSTRACT

'Tonda di Giffoni' is among the most highly appreciated Italian hazelnut (*Corylus avellana* L.) cultivars. Due to its round kernels and excellent processing quality, it was awarded a Protected Geographical Indication (PGI) from the European Union. To identify clones expressing improved nut and production qualities, a 'Tonda di Giffoni' clonal selection programme was conducted across hazelnut orchards in the Irno valley of Italy from 1995 to 2006. One hundred different clones were selected and propagated in a replicated trial under similar climate, soil, and cultural conditions. From this work, the 29 best clones were identified and from 2006 to 2008 their agronomic and pomological characteristics were observed. Microsatellite or simple sequence repeat (SSR) markers were used to successfully confirm true-to-type identity of the clones. Traits evaluated included flowering time (anthesis), bud break, suckering, trunk diameter, nut and kernel characteristics and productivity (yield). Best linear unbiased predictions for clone means and estimates of intraclass correlation coefficient were obtained using R environment, lme4 and ggplot2 packages. Five clones superior to that of the standard of 'Tonda di Giffoni' were identified in this study. Furthermore, yield and number of suckers produced showed sufficient variability to likely be exploited for breeding. The selected clones express features useful for both growers and the processing industry and will be propagated and planted in hazelnut orchards for further study and commercial production.

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1. Introduction

European hazelnut (*Corylus avellana* L.) is a major nut crop that is fifth in global importance after cashew (*Anacardium occidentale* L.), almond [*Prunus dulcis* (Miller) D.A. Webb], walnut (*Juglans regia* L.), and chestnut (*Castanea* spp.). Turkey has long been the leading producer and exporter of hazelnuts, accounting for about 71% of the world crop, followed by Italy (13%), United States (4.1%), and Spain (2.8%). Azerbaijan, Iran, Georgia, China, France, and Greece are other important producers (FAOstat, 2008). About 90% of the world crop is shelled and sold as kernels, while the remaining 10% is sold in-shell for fresh consumption (Valentini et al., 2006).

Hazelnut kernels are consumed raw, blanched or roasted or are processed for use in dairy, bakery, chocolates and other confectionery products. Kernels contain unsaturated fatty acids (linoleic, linolenic, oleic acids, palmitic and stearic) essential for human health that have been reported to decrease cholesterol levels in the blood and also control adverse effects of hypertension

(Savage et al., 1997; Amaral et al., 2006). Kernels are also rich in α -tocopherol that has been reported to lower the risk of certain chronic diseases, such as heart disease, type 2 diabetes, hypertension, cancer and may combat some of the negative effects associated with aging (Parcerisa et al., 1995; Özdemir et al., 2001; Köksal et al., 2006).

Italy has 69,685 ha planted to hazelnuts, with an average yearly production of 121,750 t in-shell (ISTAT, 2008). Four regions account for 98% of the national production: Campania, Latium, Piedmont, and Sicily, with Campania producing around one-third of the national crop. The prevailing cultivars used by the food industry are 'Mortarella' (38%) and 'San Giovanni' (37%), along with 'Tonda di Giffoni' (12%), whose production is limited to the Salerno province around the Picentini Mountains. The minor varieties 'Tonda bianca', 'Tonda rossa', 'Camponica', and 'Riccia di Talanico' are grown for fresh consumption (Piccirillo, 2002). 'Tonda di Giffoni' is much appreciated for its processing quality and has been awarded a Protected Geographical Indication (PGI) mark by the European Union as "Nocciola di Giffoni". In addition to its round kernels of excellent quality, consistent yields and resistance to pathogens and pests are its most interesting agronomic traits. However, it is prone to early bud break, which makes it susceptible to late frost damage and it is also susceptible to big bud mite attacks in mild winters.

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Turkish and European hazelnut cultivars were independently selected from local types grown over centuries (Thompson et al., 1996), with the best cultivars propagated asexually by rooted suckers or layers. Interestingly, agronomic and morphological variability can be found among clones within a single cultivar. Thus, clonal selection has been used as a means to identify improved hazelnut cultivars. Clonal selection programs have been used to identify plants expressing differences in vigour, yield, propensity to produce suckers, maturity time, nut characteristics (size, shape, kernel percent, etc.) and big bud mite resistance. Plant yield and nut morphological, physical and chemical characteristics are dependent on genotype and on its interaction with environment, including postharvest management (Bignami et al., 1999; Özdemir et al., 2001). Therefore, the controlled study of phenological and pomological traits can elucidate the relationship between genotype and environmental factors, which provides information useful to growers, breeders and the food-processing industry (Cristofori et al., 2007, 2008). In Italy, clonal selection started in the 1960s for 'Tonda Gentile delle Langhe' (Romisondo et al., 1983; Valentini et al., 2001), 'Tonda Gentile Romana' (Monastra et al., 1997), and 'Tonda di Giffoni' (Limongelli, 1983; Limongelli and Piccirillo, 2002). Also based on this method, improved clones of 'Negret' and 'Gironell' have been introduced in cultivation in Spain (Rovira et al., 1997). In Turkey, clonal selection has been used for the cultivars 'Tombul', 'Palaz', 'Kalınkara', 'Cakıldak' (İslam and Ozgüven, 2001), and 'Uzunmusa' (İslam, 2003).

The objective of this study was to characterize the agronomic, morphological, and genetic variability of 29 clonal selections of 'Tonda di Giffoni' grown under similar climate, soil, and cultural conditions to identify clones with improved nut quality and production characteristics. Microsatellite or simple sequence repeat (SSR) markers were used to verify suspected errors and to confirm true-to-type identity of the clones (Bocacci et al., 2006).

2. Materials and methods

2.1. Plant material

In 1995, 100 clones of 'Tonda di Giffoni' were selected from different hazelnut orchards growing in the Irno valley (Piacentini Mountains) of Salerno province, Italy. These plants were propagated by layering in 1998 and were grown in a nursery for 2 years to increase in size. Three plants per clone were then planted, spaced 4.5 m × 4.5 m, in a uniform comparison field located in Pignataro (Caserta province). Plants were trained to a vase shape with branching at 80 cm, irrigated for the first 2 years, and pruned

yearly to maintain their form and to eliminate suckers. Based on initial evaluations conducted in the years 1998–2000, 71 clones were eliminated and 29 were retained for further evaluation.

2.2. DNA extraction and microsatellite analysis

DNA was extracted from 0.2 g of young leaves of each clone using the modified procedure described by Thomas et al. (1993) in a Tris-EDTA-NaCl buffer containing 0.25 M NaCl, 0.2 M Tris, pH 7.6, 2.5% PVP 40,000, 0.05 M Na₂EDTA, and 0.1% β-mercaptoethanol. After purification the DNA was finally suspended in 50 μl Tris-EDTA buffer.

Ten previously reported SSR loci were used for the diversity analysis: CaT-B107, CaT-B501, CaT-B502, CaT-B503, CaT-B504, CaT-B505, CaT-B507, CaT-B508 (Bocacci et al., 2005), CaC-B020 and CaC-B028 (Bassil et al., 2005). PCR amplification was performed in a volume of 20 μL containing 50 ng DNA, 0.5 U Taq-DNA polymerase (AmpliTaq Gold, Applied Biosystems Inc., Foster City, CA), 2 μL 10× PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2 mM MgCl₂, 200 μM dNTPs and 0.5 μM of each primer. The PCR conditions included a initial denaturation step at 95 °C for 9 min, followed by 26 cycles of denaturation (30 s at 95 °C), annealing (45 s at 55 °C and 50 °C for CaT-B502), and extension (90 s at 72 °C). The final elongation step was at 72 °C for 30 min. The forward primers were labeled with a fluorochrome (6-FAM, HEX, NED or PET) and amplification products were analysed using an ABI-PRISM 3130 Genetic Analyzer capillary sequencer (Applied Biosystems). Results of the run were then processed with GeneMapper software and allele sizes were estimated using the GeneScan-500 LIZ size standard (Applied Biosystems).

2.3. Plant evaluation and statistical analysis

Agronomic and pomological traits of the 29 clones were evaluated for 3 years, starting in their fifth year of cultivation (2006–2008). Traits observed each year included flowering time (anthesis), bud break, propensity to produce suckers, yield and trunk diameter measured at 40 cm above soil level (Bioversity et al., 2008). Nut and kernel characteristics, including nut weight, nut size, shell thickness, kernel weight, kernel size, percent kernel, kernel fibre, and pellicle removal were also evaluated for each clone. Each year, three samples of 50 nuts per clone were assessed, with nut and kernel weight determined using typical well-filled nuts. The percentage of pellicle removal was estimated after blanching kernels at 120 °C for 10 min and then rubbing with a rough cloth.

Data obtained were separated using the clustering method of Scott and Knott (1974). Variance components and the intraclass

Table 1
Phenological, agronomic, and pomological trait values for 29 clones of 'Tonda di Giffoni' hazelnut. Phenological and pomological traits were calculated from three-yearly data, and yield from two-yearly records. Standard deviation was for year-to-year variation.

Trait	Unit	Mean	Range		Standard deviation		Intraclass correlation coefficient
			Min	Max	Among clones	Within clones	
Trunk diameter	mm	53.6	37	70	8.1	3.2	0.71
Leaf emergence	julian day	70	65	75	2.40	1.70	0.59
Male flowering time	julian day	350	347	354	1.40	1.90	0.42
Female flowering time	julian day	357	355	362	1.70	1.40	0.55
Suckers	N/plant	35.5	22	66	7.60	11.90	0.39
Yield per trunk section area	g/cm ²	41.1	13	104	19.50	14.50	0.57
Nut weight	g	3.0	2.7	3.4	0.00	0.30	0.00
Kernel weight	g	1.5	0.3	1.6	0.00	0.10	0.00
Percent kernel	%	48.7	45	52	0.00	3.50	0.00
Max nut diameter	mm	18.6	17.9	19.2	0.00	0.60	0.00
Max kernel diameter	mm	15.3	14.5	15.9	0.00	0.80	0.00
Shell thickness	mm	1.3	1.0	1.8	1.2	1.2	0.50

correlation coefficient, an index of genetic diversity among clones, were obtained fitting a mixed model to the three-yearly observations with random effects for the clones. Statistical and graphical summaries were made with the R environment (R Core Team, 2009) and the contributed software packages by Scott and Knott (1974), lme4 (Bates and Maechler, 2009) and ggplot2 (Wickham, 2009). To better utilize the data, two normalized indexes were developed by calculating standardized scores for agronomic and nut traits, as follows: (I) agronomic data: trunk diameter + fruit yield – number of suckers; (II) nut data: kernel percentage + kernel diameter + volume of nuts – shell thickness.

3. Results

Genetic analysis using 10 SSR loci revealed a similar genetic profile for all 29 clones that corresponded to the genetic profile of the standard 'Tonda di Giffoni' (Bocacci et al., 2006). Among the clones, significant variability was found for most phenological and agronomic traits, but not for pomological traits, except shell thickness. The intraclass correlation coefficient ranged between 39% for sucker number to 71% for trunk diameter, with 50% for shell thickness (Table 1).

Tables 1 and 2 show mean values, standard deviation, summary statistics, variance components and intraclass correlation of phenological, agronomic, nut and kernel measurements, where significant differences were present. Clones 12, 11, 25, 20 and 34 had the best nut processing scores, although they had only average scores for agronomic value (Table 2). Clone 12 was the only clone to produce a significantly higher nut yield per plant than 'Tonda di Giffoni'. Clones 6 and 25 showed the best combination of agronomic performance and nut processing quality (Fig. 2). All clones showed a high sphericity index typical of 'Tonda di Giffoni', delete from table as note in text is sufficient (Cristofori et al., 2008), which is one of the most important qualitative traits required by the confectionary industry. Clones 11 and 12 were characterised by higher nut yield than 'Tonda di Giffoni' (Table 2). Nut yield showed variability among clones with early leaf emergence (Fig. 1A) and those with large trunk

diameter (Fig. 1B). Kernel yield also showed variability among clones with large trunk diameter (Fig. 1E). Kernel yield was positively correlated with kernel weight (Fig. 1G) and shell thickness (Fig. 1H). Clones 12 and 11 were the highest yielding, the first with rather small plant and the second with larger than average original cultivar (Fig. 1B). The removal of pellicle after roasting was above 95% for all clones similar to the control 'Tonda di Giffoni'. Kernel yield and nut weight were negatively correlated (Fig. 1E).

4. Discussion

Molecular analysis of hazelnut cultivars has become a useful method in recent years. SSR markers were used to resolve cases of homonyms and synonyms, to fingerprint varieties and to search for the parents of some cultivars (Bocacci et al., 2006; Gökirmak et al., 2009). In the specific case of hazelnut clones, RAPD markers were used to determine the level of genetic diversity among 'Tonda Gentile delle Langhe' clones by Valentini et al. (2001), but genetic differences were not found. On the contrary, a 2 bp (base pairs) discrepancy was observed at loci CaT-B501 and CaT-B502 in some clones of 'Santa Maria del Gesù' and 'San Giovanni', respectively (Bocacci, personal communication). Short mutations are consistent with the stepwise mutation model proposed for microsatellite evolution (Jarne and Lagoda, 1996) and, although rare, can be observed. In grape, for example, clonal mutations at SSR loci were noted among clones (Vignani et al., 1996; Crespan, 2004) or grapevines well-known to be synonyms (Ibañez et al., 2000; Akkak et al., 2007).

In this study, no clonal mutations were observed among the 29 'Tonda di Giffoni' clones analysed, although was used a set of polymorphic SSR loci such as CaT-B501 and CaT-B502. Further analysis using a high number of SSR loci or other molecular markers, such as AFLP and SNP, could allow to identify genetic mutations among clones. Nevertheless, the SSR analysis has allowed to: (I) verify the true-to-type identity of the clones; (II) confirm that mistakes were not committed during the clonal selection. Then, breeders and growers will be able to propagate and/or to purchase material genetically certified.

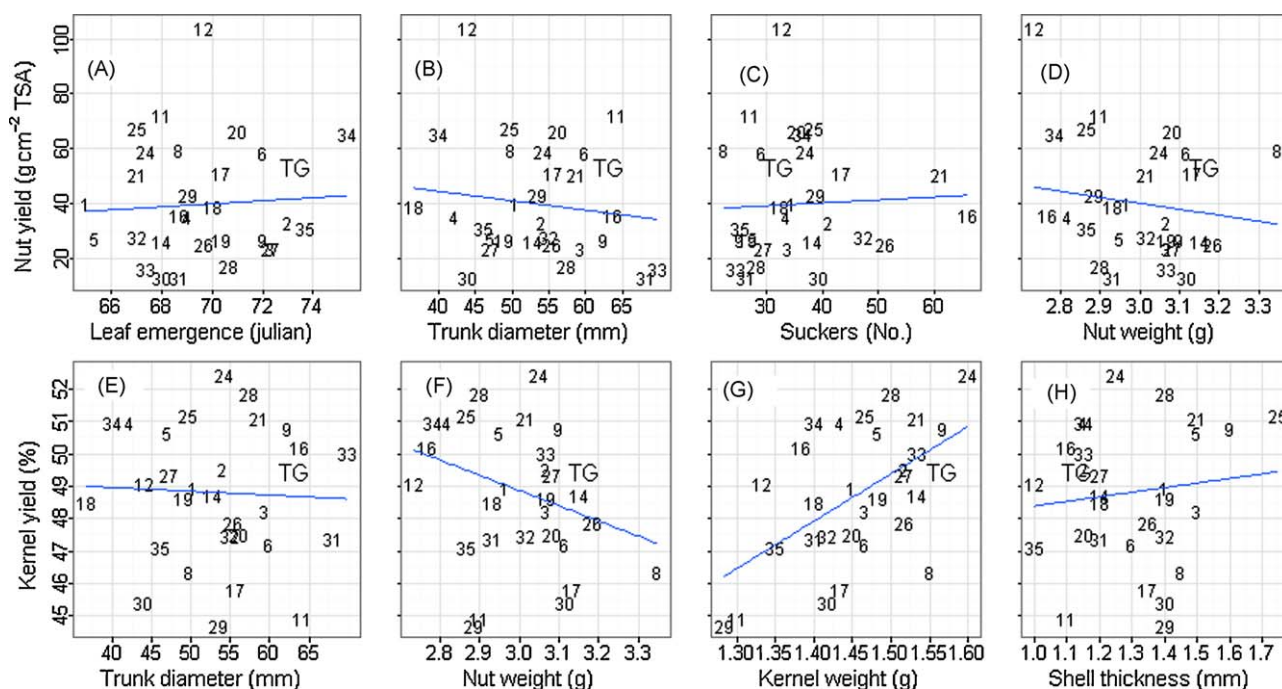


Fig. 1. Relationship between nut yield and leaf emergence (A), trunk diameter (B), suckers (C) and nut weight (D). Relationship between kernel yield and trunk diameter (E), nut weight (F), kernel weight (G) and shell thickness (H). Numbers are clone identifiers and TG is the 'Tonda di Giffoni' plant included as a control. Lines show linear and nonparametric interpolations.

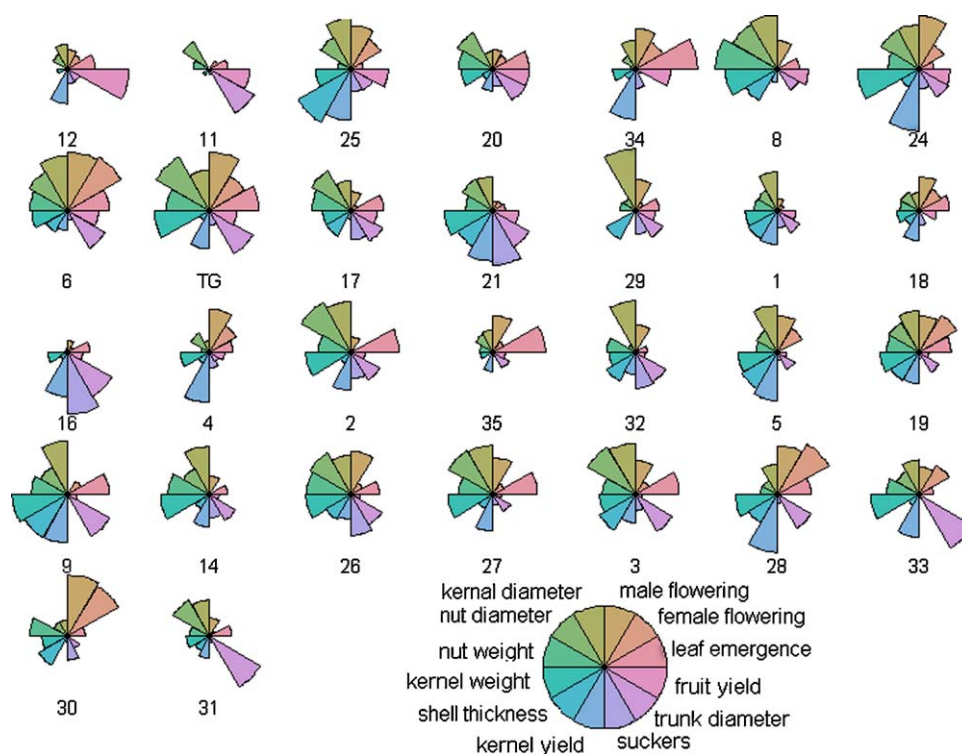


Fig. 2. Starplots of 29 'Tonda di Giffoni' clones and 'Tonda di Giffoni' standard cultivar for agronomic and nut traits.

Previous reports suggest clonal selection has produced modest results in terms of hazelnut improvement. Romisondo et al. (1983) found no differences between clones selected from a population of 'Tonda Gentile delle Langhe', but significant results were reported by Valentini et al. (2001). Small variation was found among clones selected from a population of 'Tonda Gentile Romana' (Monastra et al., 1997). Clonal selection was rated less effective in species propagated by suckers, compared with those propagated by grafting (Andreakis et al., 2002). For example, selection in the graft-propagated 'Sorrento' walnut cultivar lead to the identification of several clones with different agronomic, pomological, and molecular traits (Andreakis et al., 2002; Piccirillo, 2004).

Percent kernel is one of the most important nut processing traits. Heritability estimates of 0.92 (Thompson, 1977) and 0.87 (Yao and Mehlenbacher, 2000) have been reported for this trait. Mehlenbacher (1991) reported an average percent kernel of 55% for 'Tombul', compared to 54–60% for the same cultivar and 54–63% in 'Uzunmusa' reported by Islam (2003). Islam and Bostan (1999) reported that average of kernel percentage was 34.31–56.28%. Lower values have been reported for clones of 'Tonda Gentile Romana' (44–48%) by Monastra et al. (1997) and for clones of 'Negret' (48–51%), and 'Gironell' (39–44%) by Rovira et al. (1997). The kernel percentage of our 'Tonda di Giffoni' clones ranged between 45% and 52% and was above the average of several hazelnut cultivars. For example, clones 4, 9, 21, 24, 25 and 34 showed a kernel percentage more than 50%.

Shell thickness is correlated with high kernel percentage. A heritability of as 0.77 has been reported for this trait (Thompson, 1977). Shell thickness was found to be between 1.0 and 1.8 mm across our clones, compared to 0.86–1.05 mm in 'Tombul' and 0.75–0.93 mm in 'Uzunmusa' (Islam, 2003). These values indicate that the selected clones reported here have a shell thickness comparable to other cultivars described in the literature.

For nut weight a heritability estimate of 0.63 was determined by Yao and Mehlenbacher (2000). Across the clones studied here,

nut weight ranged from 2.7 to 3.4 g, while kernel weight ranged from 1.3 to 1.6 g with clones 8, 14 and 26 showed highest kernel and nut weight. Islam (2003) determined nut weights between 1.69 and 2.28 g for 'Tombul', between 2.05 and 2.71 g for 'Palaz', and between 1.56 and 2.34 for 'Uzunmusa'. Other studies found nut weight of 1.8 g for 'Tombul' (Mehlenbacher, 1991) and 1.40–2.01 g for 'Negret' (Rovira et al., 1997).

Pellicle removal ranged between 78% and 92%, in the high range of reported values for several cultivars: 54–92% Koksal and Okay (1997) 76–100% (Demir and Beyhan, 2000), 38–70% (Monastra et al., 1997), 50% (Mehlenbacher and Miller, 1989). These values show that the percentage pellicle removal of our selected types is higher some cultivars.

Intraclass correlation coefficient was found 0.39 for number of suckers and 0.57 for yield per trunk section area, suggesting a high variation of clones for these traits. Selected clones showed an high nut yield and a low number of suckers, two traits very important for reducing the management costs and improving hazelnuts cultivation.

In conclusion, clonal selection is an important method for the genetic improvement of hazelnut, although modest results were obtained. Nevertheless, few clonal selection programs were performed in the main hazelnut-producer Countries and on a lower number of cultivars were evaluated. Five superior clones of 'Tonda di Giffoni' were identified in this study, although none of them were outstanding for all traits. Yield and propensity to sucker showed sufficient variability to be exploited for breeding. Clones 12, 11, 25, 20 and 34 showed the best combination of percent kernel, nut shape, and yield per trunk section area and seem to be superior to the standard one. They express the best characteristics for growers and the processing industry. Then, considering that 'Tonda di Giffoni' is an important hazelnut cultivar used in new hazelnut plantations by main and new hazelnut-producer Countries, the selected clones will be propagated and planted for further observations and possible commercial production.

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