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Selection and micropropagation of valuable caper genotypes

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Abstract: The high quality of the various biotypes present in the natural or cultivated state is one of the main features for caper production. Up to now, however, no selection activity has been carried out in order to identify the most suitable accessions for providing better quality products. In this paper, we report the first results of a selection of caper genotypes characterised by significant qualitative traits. A micropropagation protocol was evaluated in order to improve and allow the multiplication of the most promising *Capparis spinosa* L. subsp. *spinosa* genotypes, selected in Salina (Aeolian Islands), Sicily (Italy), in collaboration with the most important local growers.

Keywords: *Capparis spinosa*; *in vitro* culture; selection; protocol; explants

The rapid erosion of plant genetic resources suggests the development of different biodiversity preservation methodologies, based on *in situ* and *ex situ* conservation techniques. Among these, an *in vitro* culture appears to be of great interest for an *ex situ* collection, as well as for the multiplication and conservation of a plant's germplasm (Engelmann 1991) and is one of the most promising techniques. Through the application of *in vitro* plant procedures, the Slow Growth conservation technique was developed, highlighting a series of interesting perspectives for the reduction of conservation costs, especially for perennial species (Giannì, Sottile 2015). Micropropagation is a useful technique to manage homogeneous propagation material in a short time, starting from a single explant (meristem cultures, shoot cultures, embryo cultures, isolated root cultures, callus cultures, suspension or cell cultures, protoplast cultures) and the production of pathogen-free material. The scientific research on *in vitro* cultures is discussed in many national and international scientific papers and different protocols have been developed, according to the different species. This technique is well applied in vegetable production (Butt et al. 2015), ornamental plants (Kanwar, Kumar 2008; Senapati, Rout 2008), medicinal plants (Debnath et al. 2006) and fruit trees (Sedlák, Paprštein 2008, 2011; Antonopoulou et al. 2018; Hassan, Zayed 2018). It is well known that several factors can affect the response to micropropagation and among these, the genotype is a discriminating parameter (Bobrowski et al. 1996; Scaltsoyiannes et al. 1998; Debnath 2005; Gomes et al. 2010). Considering the caper (*Capparis spinosa* subsp. *spinosa*) production, many studies have been reported on *in vitro* multiplication techniques, both with direct organogenesis and somatic embryogenesis, and advanced protocols throughout the *in vitro*

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production cycle have been developed (Rodriguez et al. 1990; Deora, Shekhawat 1995; Chalak et al. 2003; Caglar et al. 2005; Carra et al. 2011; 2012).

Despite the economic and ecological importance, *C. spinosa* is an endangered species that, if properly promoted, could make a stronger contribution to the development of local areas. Caper production is well known for the high quality of the various biotypes present in the spontaneous or cultivated state. Up to now, however, no selection activity has been carried out in order to identify the biotypes potentially suitable for providing high-quality products. Rivera et al. (2003) reported the references of caper cultivars known in the Mediterranean area, also referring to a series of controversial attributions to the various known species of the genus *Capparis* (L.). Inocencio et al. (2006) showed that most of the known cultivars belong to *Capparis spinosa* (L.) and that a large part of the varietal recognition work was carried out in the Mediterranean area and especially in Spain and Italy.

To enhance the economic value of the regional varieties of the Italian caper, it is of fundamental importance to recognise the high quality of the production and the *in vitro* culture could represent an important tool to contribute to the expansion of this crop.

In this work, a micropropagation protocol was developed on the commonly cultivated genotype in Sicily and its suitability was tested on the most promising genotypes resulting from a selection of genotypes of *Capparis spinosa* L. subsp. *spinosa* growing in Salina (Aeolian Islands, Sicily, Italy).

MATERIAL AND METHODS

Phenotypic evaluation and selection of the most valuable caper genotypes. The selection of *Capparis spinosa* (L.) subsp. *spinosa* genotypes was carried out in collaboration with caper growers of Salina (Messina Province), one of the most important islands of the Aeolian archipelago where the cultivation of the caper has a long tradition and is continuously assuming a relevant economic role. Salina is located at 38°33'40"N in the Mediterranean Sea, about 60 km from Sicily. Ten caper genotypes with evident phenotypic differences that are particularly appreciated by producers were selected.

The morphological data were recorded on 100 flower buds per genotype, collected between 10–35 cm of the fruiting shoots, to avoid collecting flower buds too young or too close to flowering. The observations were carried out every 10 days. For each flower bud, the fresh mass (g), roundness index and hardness (kg/cm^2) were reported. The diameter data were used to calculate the roundness index, the hardness was measured by a digital penetrometer (ECO GY-2) with a 2 mm tip.

Optimisation of caper micropropagation method

In the first stage, the micropropagation method for capers was optimised using a Sicilian genotype, ICAORL2. This genotype is a standard one commonly cultivated in Sicily.

Plant material and explants preparation. Young shoots were collected in the spring and the leaves were removed. The shoots were cut into nodal segments, 1 cm long, and then the surface was disinfected by immersion in 70% ethanol for 5 min and in 2% sodium hypochlorite for 20 min; after repeated washing with sterile distilled water, they were cultured on a sterile media.

Media and culture conditions. The explants were established on an MS (Murashige and Skoog 1962) solidified (8 g/l Plantagar S1000, B&V, Italy) medium supplemented with 30 g/l sucrose as a carbon source. The pH of the media was adjusted to 5.7 ± 0.1 before autoclaving. The plant growth regulators (PGRs) 6-benzylaminopurine (BAP) (Sigma B-4308), 3indole-butyric acid (IBA) (Sigma I-5.386), 3-indoleacetic acid (IAA) (Sigma I-2886), 1-naphtaleneacetic acid (NAA) (Sigma-N 0640), disinfected through a 0.22 µm filter when necessary, were added to the medium after autoclaving.

Multiplication phase. Different PGR concentrations and combinations of IBA (0.12, 0.25, and 0.50 µM) and BAP (2, 4, and 6 µM) were tested. The explants were sub-cultured into a fresh medium (the same composition) every 30 days and the cultures were maintained in a climate chamber at 25 ± 1 °C, under a 16 h day length and a photosynthetic photon flux density (PPFD) of 50 µmol/m^2/s. At the end of the multiplication phase, the explants with a shoot proliferation (%), the number of the newly grown auxiliary shoots that arose from each explant, as well as the shoot length (cm) were recorded.

Root induction phase. The actively growing shoots were excised 40 days after the *in vitro* culture initiation and used as the explants for the *in vitro* rooting phase. The shoots, 1 cm in length, were transferred on the full-strength MS medium supplemented with IBA, NAA and IAA at different concentrations (1, 5, and 10 µM) and maintained under the light growing conditions as described

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above. The rooting percentage, number of roots and root length were recorded after four weeks.

Acclimatisation phase. The rooted shoots were transferred after four weeks to Jiffy® peat pellets and maintained in Magenta GA-7 in a culture chamber at 27 ± 1 °C and at high relative humidity. After 3–4 weeks, the developed plants were exposed to a gradual reduction of humidity and after 40 days, transferred to a cold greenhouse.

Micropropagation of selected caper genotypes. In the second stage of the study on the caper micropropagation, three different genotypes (Sel. 1, Sel. 4 and Sel. 9) were tested for their suitability to the *in vitro* propagation protocol, developed on the ICAROL2 genotype. Two different initial explant types were tested:

(1) Dormant shoots, 30–40 cm in length, of the selected adult plants, were collected during the winter, rinsed with running water and Tween®20, disinfected by immersion for 10 min in 1% $HgCl_2$, placed in sterile containers containing agri-perlite and completely covered with polyethylene bags to maintain the highest humidity environment (close to 100%). The shoots were kept in a culture chamber at 25 ± 1 °C with a photoperiod of 24 h and a PPFD of 60 μmol/m^2/s. After one week, a diffuse bud-break appeared and after three weeks, the young shoots, 1.5–2.0 cm length, were further collected, disinfected as in the first stage on the micropropagation study, and used to establish the *in vitro* culture for the multiplication phase.

(2) Young shoots, 30–40 cm in length, of the selected adult plants, were collected during the spring; the leaves were removed, taking care to preserve the axillary bud. After several washings in running water and Tween®20, the shoots were disinfected for 5 min in 2% $HgCl_2$ and then cut into nodal segments to establish the *in vitro* culture.

All the explants were cultured on the MS basal medium supplemented with 20 g/l sucrose, containing 11.8 μM BAP and 12.1 μM IBA in test tubes containing 5 ml of the medium. The cultures were inoculated with a single explant and incubated for four weeks in a growth chamber at the same conditions as described for the first micropropagation experiment. The developing shoots were then cultured following the procedure described above, on the MS media supplemented with the PGR combination, considered optimal for shoot multiplication or shoot rooting, based on earlier experiments. The shoots were multiplied on the medium containing BAP at 4.0 μM and IBA at 0.5 μM.

Statistical analysis. All the statistical analyses were performed using the SPSS Statistics 22 software package (2013, IBM, Italy) for Mac. The obtained data were treated using an analysis of variance (ANOVA) and the means separated using Tukey's test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Phenotypic evaluation and selection of the most valuable caper genotypes

Morphological traits of selected genotypes. Capers, in commerce, are immature flower buds which are usually pickled in vinegar or preserved in granular salt. Semi-mature fruits and young shoots with small leaves may also be pickled for use as a condiment (Alkire 2001). Selection is one of the best plant breeding methods to enhance germplasm and develop improved cultivars (Rivera et al. 2003). In this study, the morphological data are presented for ten selected caper genotypes. Limited studies are reported on the physical properties of *Capparis* buds. It is well known that the morphological parameters are fundamental to optimise the post-harvest storage conditions and fruit processing. In Figure 1, the fresh flower mass (g) of the selected genotypes are reported. It is possible to observe three main ranges of fresh mass (0.30–0.35, 0.35–0.40 and 0.40–0.45), in order to classify the selected genotypes; in particular Sel. 1, Sel. 4 and Sel. 9 are those evidencing the higher fresh mass. Those genotypes with a roundness index of 1.3, 1.2 and 1.2 (Figure 2), respectively, showed a flower bud hardness higher than 1.8 kg/cm^2 (Figure 3).

Optimisation of caper micropropagation method

Caper propagation is commonly performed by seeds and rooted cuttings. In both cases, the propagation rate is quite low, mostly due to the high recalcitrance of the seed germination (very hard seed coat) and the poor rooting efficiency (< 50%) (Rivera et al. 2002). In recent years, *in vitro* culture and micropropagation appeared to play an important role for easily producing uniform and stable plant material. It is well known that several factors can affect the *in vitro* propagation of plants, including the genotype and the plant growth regulators (PGRs) in the culture media (Gomes et al. 2010).

In this study, a micropropagation method was developed on the commonly cultivated Sicilian genotype, ICAROL2. The influence of different concen-

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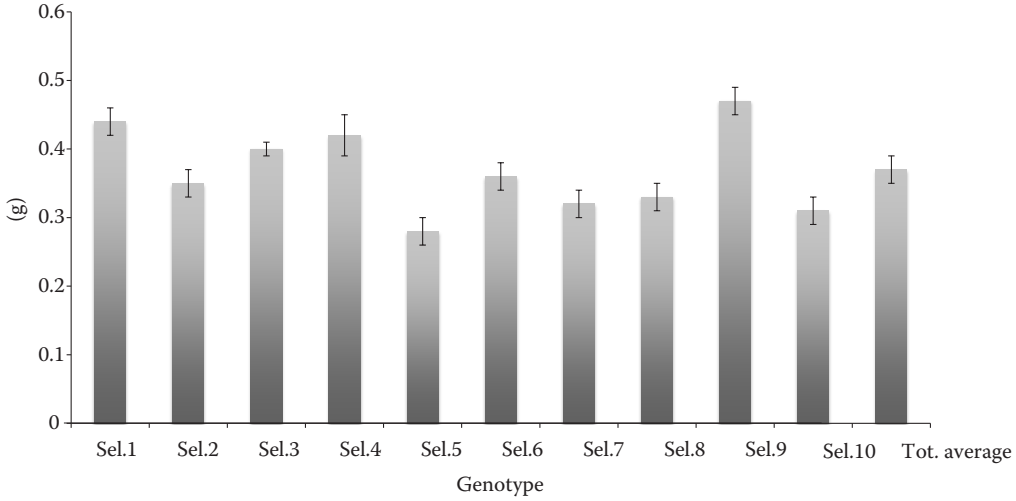

Figure 1. The fresh mass (g) of the flower buds of ten *Capparis spinosa* selected genotypes

The error bars indicate the standard deviation within the replicates

trations and combinations of auxins and cytokinins was observed after 30 days from the establishment of the *in vitro* culture and the success of the development of the nodal explants of the ICAROL2 genotype in terms of the shoot proliferation (%), number of auxiliary shoots/explant and shoot length are reported in Table 1. The *in vitro* plant regeneration was successfully obtained with all the growth regulator combinations tested, but the response of the explant showed significant differences according to the specific combination applied. BAP was demonstrated to be an efficient proliferating agent in all the tested concentrations (Rodriguez et al. 1990). Similarly, Abbas and Qaiser (2010) have shown that different BAP concentrations in a culture medium had a significant effect on the shoot regeneration frequency (65%) and on the number of explant buds in *Cadaba heterotricha* (Capparaceae).

In our study, the shoot proliferation varied into a range with a min. of 55.4% (BAP 2 µM + IBA 0.5 µM) to a max. of 98.1% (BAP 4 µM + IBA 0.5 µM). The highest proliferation capacity (91%) observed with the maximum concentration of BAP (6 µM) was in association with the lowest concentration of IBA (0.12 µM). This combination also positively influenced the response in terms of the number of shoot/explants (8.7) and the shoot length (2.7). When increasing the IBA concentrations in the

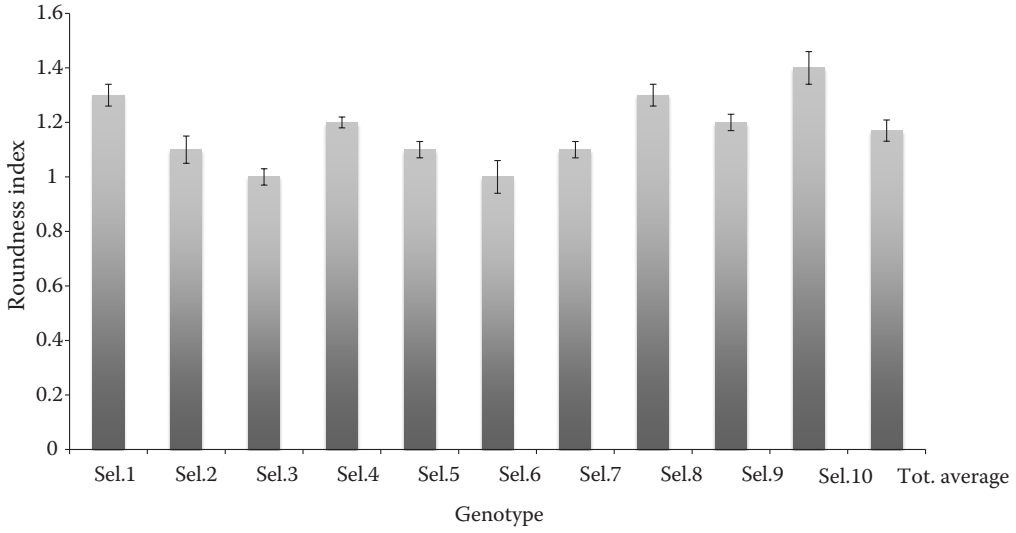

Figure 2. The roundness index of the flower buds of ten *Capparis spinosa* selected genotypes

The error bars indicate the standard deviation within the replicates

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Table 1. The effects of the different PGR treatments on the in vitro nodal explants of the *Capparis spinosa* ICAROL2 genotype

IBA concentration (μM)	BAP concentration (μM)	Explants with shoot proliferation (%)	Auxiliary shoots/ explant (No.)	Shoot length (cm)
0.12	2	83.3 ± 1.0^{b}	8.3 ± 2.2^{a}	2.3 ± 0.4^{a}
	4	65.1 ± 4.0^{c}	6.1 ± 2.1^{b}	1.2 ± 0.3^{b}
	6	91.0 ± 3.0^{a}	8.7 ± 1.6^{a}	2.7 ± 0.9^{a}
0.25	2	80.2 ± 4.1^{a}	5.5 ± 0.7^{b}	2.1 ± 0.1^{ns}
	4	59.4 ± 5.1^{c}	6.5 ± 2.1^{b}	2.1± 0.5^{ns}
	6	70.2 ± 4.1^{b}	8.1 ± 1.9^{a}	1.9 ± 0.2^{ns}
0.50	2	55.4 ± 5.2^{b}	5.2 ± 1.6^{b}	2.1 ± 0.1^{ns}
	4	98.1 ± 0.1^{a}	8.8 ± 1.9^{a}	2.1± 0.5^{ns}
	6	60.2 ± 4.1^{b}	7.9 ± 2.2^{a}	2.0 ± 0.3^{ns}

The means ± standard deviation in the columns for each IBA concentration, separately followed by different letters is significantly different at $P \leq 0.05$ according to Tukey's test; IBA – 3indole-butyric acid; BAP – 6-benzylaminopurine

media (0.50 μM), the most effective results were achieved in association with a medium concentration of BAP (4 μM) (98.1 % shooting proliferation and 8.8 shoots/explant).

These explants were cultured in a rooting medium to induce the roots (Table 2). Concerning the rooting percentage of the three auxins tested, IBA was found to be the most effective treatment with all the tested concentrations. The best rooting rate (93.4%) was achieved with IBA 5 μM. The maximum root length (2.9 cm) corresponding to this condition had 2.6 roots per plant. The *Capparis spinosa* shoots failed to induce roots or root initials with concentrations above 1 μM of NAA and 10 μM of IAA; in fact, under these growing conditions a callus formation was observed.

Micropropagation of the selected caper genotypes

The micropropagation protocol developed on the genotype ICAROL2 was validated on the most promising genotypes resulting from the phenotypic selection. In particular, Sel. 1, Sel. 4 and Sel. 9 were the genotypes with the highest qualitative performances in terms of the morphological traits.

No statistical differences in the efficiency of the shoot development from the two types of initial explants (dormant and young shoots) were observed, however, the average results obtained from the dor-

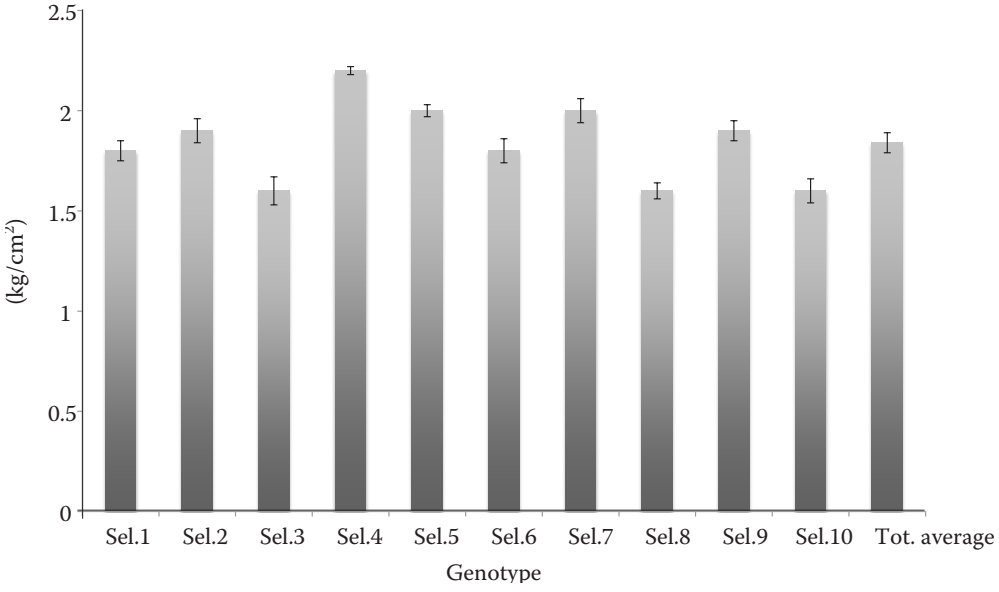

Figure 3. The flower bud hardness of ten *Capparis spinosa* selected genotypes

The error bars indicate the standard deviation within the replicates

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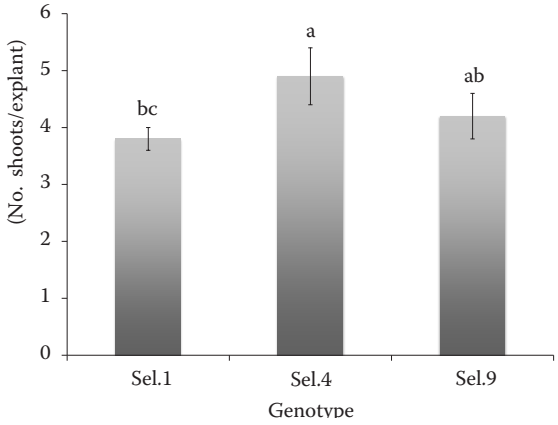

Figure 4. The shoots/explant *in vitro* propagation of three selected *Capparis spinosa* genotypes

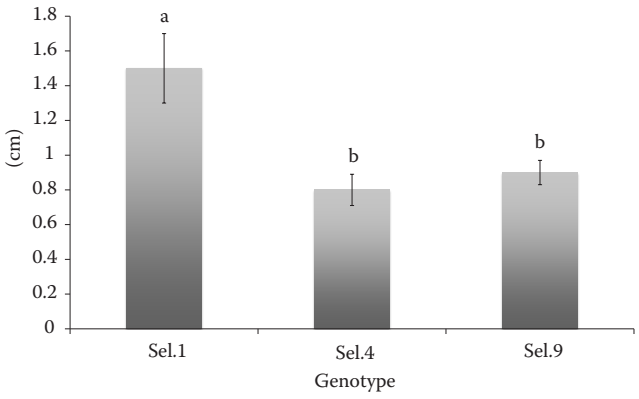

Figure 5. The shoot length *in vitro* propagation of three selected *Capparis spinosa* genotypes

The error bars indicate the standard deviation within the replicates; the means followed by the different letters are significantly different according to Tukey's test at $P \leq 0.05$

mant shoots are reported in Figure 4 and Figure 5. The use of dormant shoots is of particular interest as a valid alternative during dormancy, inducing shoots able to be directly established to the proliferation phase, and to overcome the plant regeneration from flower explants, as described in Carra et al. (2012); thus, considerably reducing the time and costs of the micropropagation process.

All three genotypes showed good suitability to the *in vitro* propagation protocol. Furthermore, when the selected genotypes were tested, the analysis of the data evidenced a genotype-effect on the multiplication rate both in terms of the shoot length and the number of shoots, with some genotypes, i.e., Sel. 4, showed the highest multiplication rate generating up to almost five shoots per explant, while Sel. 1 generated less than four. Sel. 1 produced the longest shoots, 1.5 cm in length while for the other two genotypes the average length was less than 1 cm (Figure 5).

The results showed the interesting performance of the *in vitro* introduction protocols of the species, with a moderate genotype influence.

Although the described micropropagation method was interesting and functional for the three selected genotypes, continuous adjustments in order to maintain high efficiency need to be considered to overcome the recalcitrant phenomena.

CONCLUSION

In recent years, *in vitro* culture and micropropagation have played an important role in producing uniform and stable plant material and will undoubtedly contribute to the further growth of the sector. The suitability of a micropropagation protocol

Table 2. The effects of the different treatments on the *in vitro* shoot rooting of the *Capparis spinosa* ICAROL2 genotype

Growth regulator (μM)	Rooting (%)	Root number	Root length (cm)
IBA 1	37.8 ± 0.3^{d}	3.3 ± 0.7^{a}	1.2 ± 0.1^{c}
IBA 5	93.4 ± 0.2^{a}	2.6 ± 0.3^{a}	2.9 ± 0.3^{a}
IBA 10	68.4 ± 0.2^{b}	2.6 ± 0.3^{a}	2.4 ± 0.2^{a,b}
NAA 1	68.2 ± 0.4^{b}	2.0 ± 0.3^{b}	1.2 ± 0.1^{c}
NAA 5	–	–	–
NAA 10	–	–	–
IAA 1	55.2 ± 0.3^{c}	2.5 ± 0.5^{a,b}	2.0 ± 0.2^{b}
IAA 5	25.2 ± 0.3	3.3 ± 0.3^{a}	1.4 ± 0.2^{c}
IAA 10	–	–	–

The means ± standard deviation in the columns followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test

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was tested on a selection of *Capparis spinosa* L. subsp. *spinosa* genotypes from the Aeolian Islands. Among all the PGR combinations tested on the three selected caper genotypes (Sel. 1, Sel. 4 and Sel. 9), the highest shoot proliferation performance was observed with the maximum BAP concentration and the lowest IBA concentration. The results described here are preliminary and need to be supported by a more detailed analysis, through the evaluation of the tested genotypes during the rooting and acclimatisation phases, in order to understand if this protocol can be proposed for an efficient clonal propagation activity of genotypes, directly derived from the genetic breeding in advanced nurseries.

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