THE MAIN RESULTS IN ACCLIMATIZATION AND BREEDING OF *BENINCASA HISPIDA*

Costel VÎNĂTORU¹, Adrian George PETICILĂ², Elena BARCANU¹, Bianca MUȘAT¹, Camelia BRATU¹, Bianca TĂNASE^{1, 2}, Ovidia-Loredana AGAPIE¹, Ion GHERASE¹, Geanina NEGOȘANU¹

¹Vegetable Research Development Station Buzău, 23 Mesteacănului Street, Buzău, Romania ²University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: costel vinatoru@yahoo.com

Abstract

Benincasa hispida, known as winter melon, name inspired by the fruit shape similar to melon and also to the fact that fruits are kept very well after harvest, over winter time. The Breeding and Biodiversity Laboratory from VRDS Bužau has taken into study this species after year 1996. The research began with purchase of genetic material from various area of origin, followed by acclimatization of the species. After year 2005, valuable genotypes have been subject to intensive breeding work and the research completed so far with obtaining of three genotypes (G1, G2, G3) with distinct phenotypic expressiveness, especially in terms of fruit shape and size. G1 has elongated, cylindrical, rounded fruits at both ends, with an average fruit mass of 3.39 kg; G2 has round fruits, slightly ovoid, with an average fruit mass of 8.77 kg; G3 has large, round fruits with a pronounced depth on the top and with an average fruit mass of 14.42 kg. The G1 genotype has been proposed for patenting and from 2021 it will be registered in the Official Catalogue of Românian Crop Plants.

Key words: Cucurbitaceae, phenotype, wax gourd, winter melon.

INTRODUCTION

Benincasa hispida (Thunb.) belongs to Cucurbitaceae family and is also known as wax gourd, winter melon, Chinese watermelon, Kundur, tallow gourd, winter gourd and white pumpkin. The area of origin is not known exactly, but is probably native in Japan and Java and is widely cultivated or less throughout India and in warm countries from Asia. In Asian communities *B. hispida* is a popular vegetable crop both for nutritional and medical purposes (Nimbal et al., 2011; Zaini et al., 2011). The fruit may be used raw in salads, cooked with meat or different kind of vegetables (Stephens, 2012). In traditional medicine the plant was used in various complains such as respiratory disease, heart disease, diabetes mellitus, urinary diseases, gastrointestinal problems, menstrual disorders, fever, insanity, epilepsy, schizophrenia and other psychological disorders (Blatter et al., 1975; Joshi, 2000; Sharma and Medhyadi, 2005; Jayasree et al., 2011). Nowadays, pharmacological studies revealed that the plant exerted many pharmacological activities. Studies conducted by Huang et al. (2004) and Roy et al. (2007) on fruit of winter melon revealed that its juice and extract have antioxidant activity especially on human tissues like liver and brain. Roy et al. (2008) discovered that a dose of 400 mg/kg body weight, of B. hispida fruit seemed to have a protective effect on Alzheimer's disease. Other studies revealed anti-compulsive effect (Girdahar et al., 2010), anti-ulcer effects (Grover et al., 2001; Rachchh and Jain, 2009), anti-inflammatory effects (Cuzzocrea et al., 2001; Gill et al., 2010; Shetty et al, 2008), antiobesity effects (Kumar and Vimalavathini, 2004; Zhang, 1996), anti-diarrheal agent (Mathad et al., 2005). The major constituents of B. hispida fruits are flavonoids, glycosides, sacchrides, proteins, carotenes. vitamins. minerals, volatile oils and uronic acid (Yoshizumi et al., 1998; Rana and Suttea, 2012; Chidan et al., 2012; Mandana et al., 2012, Busuioc et al., 2020). Zaini et al. (2011) recommends that due to high nutritional value and its growing demand, winter melon fruit should be used in different food commodities such as jams, juices, beverages, cakes and ice creams for value-addition.

Benincasa hispida is a particularly valuable species in term of its use as a rootstock. The species is compatible with most melon and cucumber cultivars. At the same time, the plant has a vigorous and well-developed root system which explores the deep layers of the soil and also has a genetic resistance to specific soil pests (Vînătoru et al., 2019).

In a survey made by Dobre and Toma in 2013, regarding the perception of *Benincasa* fruit it was shown that with a proper promotion the vegetable can be introduced in Romanian consumer's behaviour.

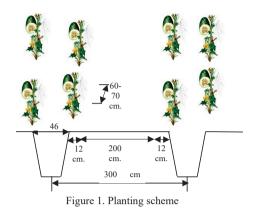
The aim of this work was to acclimatize the species in Romania and to obtain new genotypes with distinct phenotypic characteristics.

MATERIALS AND METHODS

The Breeding and Biodiversity Laboratory from Vegetable Research Development Station Buzău has taken into study this species after year 1996. The research began with purchase of genetic material from various area of origin, followed by acclimatization of the species. After year 2005, valuable genotypes have been subject to intensive breeding work and the research completed so far with obtaining of three genotypes (G1, G2 and G3) with distinct phenotypic expressiveness, especially in terms of fruit shape and size. The breeding method used was repeated individual selection. The seedlings were produced in alveolar pallets with 70 cubes. After 60 days, the seedlings were planted in the greenhouse, in first decade of April.

The planting scheme used in the greenhouse was 70 cm between plants and 300 cm between rows (Figure 1).

The special care works were the one specific to Cucurbitaceae species. The plants were vertically support. A particular attention has been paid to isolation distance between genotypes in order to prevent their impurity, knowing that the species is allogamous and preferred by the insect, especially by the bees. During the vegetation period, phenological, biometrical observations were performed. In order to characterize the genotypes IPGRI descriptors were used and biometric determinations were made.



RESULTS AND DISCUSSIONS

Winter melon is a monoecious vine, easier to grow than any other plants from Cucurbitaceae family. It can be grown spread on the ground/soil or trained to climb a support. The research has shown that the best results are obtained when the crop is vertically supported. A special attention should be paid to G2 and G3 genotypes because it has very large fruits that can detach easily from plant, reason why fruits needs support. The stem is hairless at the base, but as it grows it becomes pubescent and is covered with coarse hair. Foliage is simple, with large leaves of different length varying from genotype to genotype. The leaf width varies from 27.3 cm (G1) to 37.2 cm (G3). More details about phenotypic expressiveness of the leaves can be found in Tables 1-3.

The young leaves and flower buds can be steamed and eaten in different recipes. The flowers are solitary, yellow coloured and five parted. Male flowers appear first and female flowers bear miniature fruit.

The male flower diameter is large varying from 9.6 cm (G1) to 10.5 cm (G3), in return, the female flowers are smaller, with a diameter ranging from 8.1 cm (G1) to 8.7 cm (G3). Regarding petiole length there are also some differences, the male flower has a length varying from 22.9 cm (G1) to 25.7 cm (G3), while the female flowers registers values between 9.7 cm (G1) and 11.5 m (G3).

Table 1. Main characteristics of G1 leaf

| Character | $\overline{x}\pm SD$ | $\overline{\mathbf{X}}$ | SD | CV% |
|-----------------------|----------------------|-------------------------|----|-------|
| Leaf length (cm) | 19-29 | 24 | 5 | 20.83 |
| Leaf width (cm) | 27.3-37.1 | 32 | 5 | 15.62 |
| Petiole length (cm) | 11-17 | 14 | 3 | 21.42 |
| Petiole diameter (mm) | 7.5-9.5 | 8.5 | 1 | 11.76 |

| Character | $\overline{x}\pm SD$ | $\overline{\mathbf{X}}$ | SD | CV% |
|-----------------------|----------------------|-------------------------|-----|-------|
| Leaf length (cm) | 25.0-26.2 | 25.6 | 0.6 | 2.34 |
| Leaf width (cm) | 34.2-35.4 | 34.8 | 0.6 | 1.72 |
| Petiole length (cm) | 15.2-17.6 | 16.4 | 1.2 | 7.31 |
| Petiole diameter (mm) | 7.8-10.6 | 9.2 | 1.4 | 15.21 |

Table 2. Main characteristics of G2 leaf

| Table 3. | Main | characteristics | of G3 | leaf |
|----------|------|-----------------|-------|------|
|----------|------|-----------------|-------|------|

| Character | $\overline{x}\pm SD$ | $\overline{\mathbf{X}}$ | SD | CV% |
|-----------------------|----------------------|-------------------------|-----|------|
| Leaf length (cm) | 24.6-28.2 | 26.4 | 1.8 | 6.81 |
| Leaf width (cm) | 34.0-37.2 | 35.6 | 1.6 | 4.49 |
| Petiole length (cm) | 16.0-18.4 | 17.2 | 1.2 | 6.97 |
| Petiole diameter (mm) | 9.2-10.4 | 9.8 | 0.6 | 6.12 |

Mature fruits of *Benincasa hispida* are covered with a waxy coat which allows them long-term storage. The fruit shape may be elongated, ovoid or round (Figures 2 and 3).



Figure 2. Immature and mature fruit of G2



Figure 3. Mature fruit of G1 (left) and G3 (right)

G1 has elongated, cylindrical, rounded fruits at both ends; G2 has round fruits, slightly ovoid; G3 has large, round fruits with a pronounced depth on the top.

The first fruit reached technical maturity in the third decade of June and physiological maturity, in the last decade of September.

Fruit mass range from 2.390 kg (G1) to 16.840 kg (G3) and more details about the main characteristics of fruit genotypes can be found in Tables 4-6.

Table 4. Main characteristics of G1 fruit

| Character | $\overline{x}\pm SD$ | x | SD | CV% |
|---------------------------------|----------------------|-------|-------|-------|
| Fruit mass (kg) | 2.390 - 4.390 | 3.390 | 1.000 | 29.49 |
| Fruit length (cm) | 31-39 | 35 | 4.0 | 11.42 |
| Median fruit diameter (cm) | 11.515.5 | 13.5 | 2.0 | 14.81 |
| Pulp thickness (cm) | 3.1-3.7 | 3.4 | 0.3 | 8.82 |
| Pericarp thickness (cm) | 2.5-3.5 | 3 | 0.5 | 16.66 |
| Core thickness (cm) | 5.5-6.6 | 6 | 0.5 | 8.33 |
| Inner hollow median diameter | 6.2-7.4 | 6.8 | 0. | 8.82 |
| Diameter of blossom end (mm) | 8-12 | 10 | 2.0 | 2.0 |
| Blossom end fruit dent (mm) | 2-4 | 3 | 1.0 | 33.33 |
| Stem end fruit dent (mm) | 0-2 | 1 | 1.0 | 100.0 |

Table 5. Main characteristics of G2 fruit

| Character | $\overline{x}\pm SD$ | $\overline{\mathbf{X}}$ | SD | CV% |
|---------------------------------|----------------------|-------------------------|-----|-------|
| Fruit mass (kg) | 8.210- 9.330 | 8.770 | 560 | 6.38 |
| Fruit length (cm) | 28-40 | 34 | 6 | 17.64 |
| Median fruit diameter (cm) | 24-32 | 28 | 4 | 14.28 |
| Pulp thickness (cm) | 3.6-4.8 | 4.2 | 0.6 | 14.28 |
| Pericarp thickness (cm) | 1.5-2.5 | 2 | 0.5 | 25.0 |
| Core thickness (cm) | 6.8-7.6 | 7.2 | 1.6 | 22.22 |
| Inner hollow median diameter | 17-21 | 19 | 2 | 10.52 |
| Diameter of blossom end (mm) | 13-19 | 16 | 3 | 18.75 |
| Blossom end fruit dent (mm) | 8-12 | 10 | 2 | 20.0 |
| Stem end fruit dent (mm) | 12-16 | 14 | 2 | 14.28 |

The pericarp thickness is associated with shelflife of fruit and the highest value was recorded by G1, with a thickness of 3 cm, followed by G2 and G3 with a thickness of 2 cm.

| Character | $\overline{x}\pm SD$ | $\overline{\mathbf{X}}$ | SD | CV% |
|---------------------------------|-----------------------|-------------------------|-------|-------|
| Fruit mass (kg) | 12.000 - 16.840 | 14.420 | 2.420 | 16.78 |
| Fruit length (cm) | 31-41 | 36 | 5 | 13.88 |
| Median fruit diameter (cm) | 28-38 | 33 | 5 | 15.15 |
| Pulp thickness (cm) | 5.1-5.7 | 5.4 | 0.3 | 5.55 |
| Pericarp thickness (cm) | 1,5-2,5 | 2 | 0.5 | 25.0 |
| Core thickness (cm) | 9.8- 11.4 | 10.6 | 0.8 | 7.54 |
| Inner hollow median diameter | 21.5- 23.5 | 22.5 | 1.0 | 4.44 |
| Diameter of blossom end (mm) | 19-25 | 22 | 3 | 13.63 |
| Blossom end fruit dent (mm) | 28-40 | 34 | 6 | 17.64 |
| Stem end fruit dent (mm) | 16-21 | 18 | 2 | 11.11 |

Table 6. Main characteristics of G3 fruit

The flesh of mature fruit is white, spongy and juicy. The edible part of mature fruit is considered the core. The core thickness has the highest value registered by G3 with a mean of 10.6 cm, followed by G2 with a mean of 7.2 cm and G1 with a mean of 6.05 cm.

The number of fruits per plant depends when the harvest is made. If the fruits are harvested at technical maturity genotype G1 has 20-35 fruits/plant.

If the fruits are harvested at psychological maturity, the number of fruits per plant is lower, therefore 10-12 fruits. The G2 genotype has a yield potential of 15-20 fruits per plant when harvest at technical maturity and 8-10 fruits/plant when harvested at psychological maturity. The G3 genotype has larger fruits, and the number of fruits per plant is lower, it was noted that when fruits are harvested at technical maturity the plant can bear 10-15 fruits, but when the fruits are left to be harvested at physiological maturity, the number of fruits is lower, thus 6-8 per plant.

Longitudinal section of the fruit is presented in Figures 4-5.

Winter melon seeds contains high amount of fatty acids 24.3%, saturated fatty acids represents 75.38% and unsaturated fatty acid (Mandana et al., 2012). The seeds have different shapes (Figure 6), depending on the shape and type of fruit.



Figure 4. Longitudinal section of G2 (left) and G1 (right)



Figure 5. Longitudinal section of G3

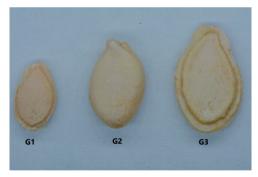


Figure 6. Seeds of B. hispida

The average seed length varies from 8.1 mm on G1, followed by G2 with 11.1 mm and G3 with 13.3 mm. Seed width also range from 4.2 mm (G1), to 6.3 mm (G2) and 8.4 mm (G3). Mass of thousands seeds weighs 23.34 g (G1), followed by 26.83 g (G2) and 33.04 g (G3). The seed mass in fruit differs depending on genotype as it follows, G1 weighs 23.83 g, G2 weighs 29.60 g and G3 weighs 126.26 g.

During vegetation period it was noted that no serious insects or disease problems were recorded and we recommend winter melon crop to be grown also in organic farming.

CONCLUSIONS

The research undertaken so far certifies that the species can be successfully grown throughout of Romania. During the research, three genotypes with distinct phenotypic expressiveness were obtained, of which, G1 will be registered in the Official Catalogue of Romanian Crop Plants.

Genotypes G1 and G3 are proposed for registration for year 2022. The Vegetable Research Development Station Buzau has promoted the species with its health benefits and the demand for seeds and seedlings has increased significantly from year to year among growers and also the consumers demand.

ACKNOWLEDGEMENTS

This work was supported by one founding source a grant of the Romanian Ministry of Research and Innovation, CCCDI – UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0850/ contract 14 PCCDI /2018, within PNCDI III.

REFERENCES

- Blatter E, Caius JF & Mhaskar KS (1975). Indian Medicinal Plants, 2nded, Vol 2, Bishen Singh Mahendra Palsingh, pp 1126-1128.
- Busuioc, A. C., Botezatu, A. V. D., Furdui, B., Vinatoru, C., Maggi, F., Caprioli, G., & Dinica, R. M. (2020). Comparative Study of the Chemical Compositions and Antioxidant Activities of Fresh Juices from Romanian Cucurbitaceae Varieties. Molecules, 25(22), 5468.
- Chidan Kumar CS, Mythily R, & Chandraju S. (2012). Extraction and mass characterization of sugars from ash gourd peels (*Benincasa hispida*). *Rasayan J Chem*, 5(3): 280-285.
- Cuzzocrea, S., Riley, D. P., Caputi, A. P., & Salvemini, D. (2001). Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. *Pharmacological Reviews*, 53, 135-159.
- Dobre C. & Toma, E. (2013). The romanian consumer perception on the *Benincasa hispida* fruit. *Scientific Papers: Management, Economic Engineering in Agriculture & Rural Development*, 13(2).
- Enache Florin, Sorin Matei, Gabi-Mirela Matei, Ionuț Ovidiu Jerca, Elena Maria Drăghici (2019). Stimulation of plant growth and rhizosphere microbial communities by treatments with structured

water, *Scientific Papers. Series B, Horticulture*. Vol. LXIII, No. 1, pag. 365-370, 2019, Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.

- Gill, N. S., Dhiman, K., Bajwa, J., Sharma, P., & Sood, S. (2010). Evaluation of free radical scavenging, anti-inflammatory and analgesic potential of *Benincasa hispida* seed extract. *International Journal of Pharmacology*, 6, 652-657.
- Girdhar, S., Wanjari, M.M., Prajapati, S. K., & Girdhar, A. (2010). Evaluation of anti-compulsive effect of methanolic extract of *Benincasa hispida* Cogn. fruit in mice. *Acta Poloniae Pharmaceutica*-Drug Research, 67, 417-421.
- Grover, J. K., Adiga, G., Vats, V., & Rathi, S. S. (2001). Extracts of *Benincasa hispida* prevent development of experimental ulcers. *Journal of Ethnopharmacology*, 78, 159-164.
- Huang, H. Y., Huang, J. J., Tso, T. K., Tsai, Y. C., & Chang, C. K. (2004). Antioxidant and angiotensionconverting enzyme inhibition capacities of various parts of *Benincasa hispida* (wax gourd). *Nahrung*, 48, 230-233.
- Jayasree T, Kishore K, Vinay M, Vasavi P, Chandrasekhar N, Manohar VS & Dixit R., (2011) Evaluation of the Diuretic effect of the chloroform extract of the *Benincasa hispida* rind (Pericarp) Extract in Guinea-pigs. *Journal of Clinical and Diagnostic Research*, 5(3): 578-582.
- Joshi S. (2000). Benincasa hispida In: Joshi S, editor. Medicinal Plants. p 152.
- Kumar, A., & Vimalavathini, R. (2004). Possible anorectic effect of methanol extract of *Benincasa hispida* (Thunb.) Cong, fruit. *Indian Journal of Pharmacology*, 36, 348-350.
- Matei Ovidiu, Hayder Adil Abdulrazzaq Al-Kinani, Aurora Dobrin, Ovidiu Ionuţ Jerca, Elena Maria Drăghici (2019). Effects of organic fertilizers on the growth seedlings of asparagus - pag. 117-122, *Scientific Papers. Series B, Horticulture.* Vol. LXIII, No. 2, 2019, Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.
- Mandana, B, Russly AR, Farah ST, Noranizan MA, Zaidul IS & Ali G. (2012). Antioxidant activity of winter melon (*Benincasa Hispida*) seeds using conventional soxhlet extraction technique. *International Food Research Journal*, 19(1): 229-234.
- Mathad, V. S. B., Chandanam, S., Setty, S. R. T., Ramaiyan, D., Veeranna, B., & Lakshminarayanasettry, A. B. V. (2005).
 Antidiarrheal evaluation of *Benincasa hispida* (Thunb.) Cogn. Fruit extract. *Iranian Journal of Pharmacology and Therapeutics*, 4, 24-27.
- Nimbal SK, Venkatrao N, Ladde S & Pujar B. (2011). Anxiolytic evaluation of *Benincasa hispida* (Thunb) Cogn. fruit extracts. *International Journal of Pharmacy and Pharmaceutical Science Research*, 1(3), 93-97.
- Rachchh, M. A., & Jain, S. M. (2009). Gastroprotective effect of *Benincasa hispida* fruit extract. *Indian Journal of Pharmacology*, 40, 271-275.

- Rana S & Suttee A. (2012). Phytochemical investigation and evaluation of free radical scavenging potential of *Benincasa hispida* peel extracts. *International Journal of Current Pharmaceutical Review and Research*, 3(3):43-46.
- Roy, C., Ghosh, T. K., & Guha, D. (2007). The antioxidative role of *Benincasa hispida* on colchicine induced experimental rat model of Alzheimer's disease. *Biogenic Amines*, 21, 1–2.
- Roy, C., Ghosh, T. K., & Guha, D. (2008). Dose dependent activity of *Benincasa hispida* on colchicines induced experimental rat model of Alzheimer's disease. *International Journal of Pharmacology*, 4, 237-244.
- Sharma P.V. & Medhyadi Varg (2005). In The Dravya Gun-Vijnana Vegetable Drugs. Sharma PV (ed.) Chaukhambha Bharti Academy, Varanasi 2005, p 14.
- Shetty, B. V., Arjuman, A., Jorapur, A., Samanth, R., Yadav, S. K., Valliammai, N. (2008). Effect of extract of *Benincasa hispida* on oxidative stress in rats with Indomethacin induced gastric ulcers. *Indian Journal of Physiology and Pharmacology*, 52, 178-182.

- Stephens JM. (2012). Gourd, Wax Benincasa hispida (Tzmmmhunb.), Cogn. University of Florida. http://edis.ifas.ufl.edu. Wu CM, Liou SE, Chang YH and Chiang W. Volatile compounds of the wax gourd (Benincasa hispida, Cogn) and a wax guard beverage. J Food Sci 1987;52:132-4
- Vînătoru C., B. Muşat & C. Bratu.(2019). Tratat de legumicultură specială. Editura ALPHA MDN, Buzău, 325-327.
- Yoshizumi S, Murakami T, Kadoya M, Matsuda H, Yamahara J & Yoshikawa M. (1998). Medicinal foodstuffs. XI. Histamine release inhibitors from wax gourd, the fruits of *Benincasa hispida* Cogn. *Yakugaku Zasshi*, 118:188-192.
- Zaini NAM, Anwar F, Abdul Hamid A & Saari N. (2011). Kundur [*Benincasa hispida* (Thunb.) Cogn.]: A potential source for valuable nutrients and functional foods. *Food Res Int*; 44:2368-2376.
- Zhang, S. Y. (1996). Cosmetic and medicinal effects of the fruit of *Benincasa hispida*. *Zhongguo Mingjian Liofa*, 4, 44-49.