Diversity and Valorization of Local Genetic Resources of *Cucurbita* in Tunisia

Hela Chikh-Rouhou
Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB), LR21AGR03, University of Sousse, Sousse 4042, Tunisia. Email: hela.chikh.rouhou@gmail.com

Imen Tlili, Imen Henane, and Riadh Ilahy
University of Carthage, Laboratory of Horticulture, National Agricultural Research Institute of Tunisia (INRAT), Rue Hédi Karray 1004 El Menzah, Tunisia

Ana Garcés-Claver
Department of Plant Science, Agrifood Research and Technology Centre of Aragon (CITA). Avda. Montañana 930, 50059, Zaragoza, Spain; AgriFood Institute of Aragon – IA2 (CITA-University of Zaragoza), Zaragoza, Spain

The genus *Cucurbita* (2n = 40, Whitaker and Robinson, 1986), incorporating pumpkin and squashes or vegetable marrows, is a valuable genus of the Cucurbitaceae family with 21 species, five of which (*C. moschata* Duchesne, *C. pepo* L., *C. maxima* Duchesne, *C. argyrosperma* Huber and *C. ficifolia* Bouché) are cultivated (Khoury et al., 2019). Pumpkins and squash are easily grown in tropical, sub-tropical, warm-temperate and temperate climates, and in cool-temperate climates can be successfully grown if provided early-season protection from low temperatures (Paris, 2018). Pumpkin now occupies a prominent place among vegetables owing to its high productivity, nutritive value, good storability, long period of availability, and better transport potentialities (Hazra et al. 2007). They are generally cultivated for their fruits and sometimes for their oil seeds, flowers, and leaves (Caili et al. 2006).

Cucurbita spp. have versatile fruits with fleshy shell, seeds, and even edible flowers. The immature fruits of various *Cucurbita* have been used for culinary purposes in different parts of the world. Ripe pumpkin fruits can be boiled, baked, or steamed (Roberts, 2006). The benefits of *Cucurbita* fruits are very important in terms of human health and are good for digestion and supplying energy (Bisognin, 2002). Raw or roasted pumpkin and squash seeds are used as a snack food for human consumption in many countries all over the world. A seed extract has been reported to have antidiabetic, antitumor, antibacterial, anticancer, and antioxidant activities. It has also been found to have strong hypotriglyceridemic and serum cholesterol-lowering effects (Tlili et al. 2020; Caili et al. 2006). Pumpkin has high nutritional value essential for food security (Gbemenou et al. 2022). Despite many benefits, it is still considered an orphan crop in Africa and very little information is available on the potential and production of pumpkin in Africa due to neglect by researchers and improvement programs (Gbemenou et al. 2022).

In Tunisia, pumpkin (*Cucurbita* spp.) has significant economic importance especially in subsistence agriculture because of its vigor, nutritional values, and long shelf life. There are no improved cultivars of squash and pumpkin in Tunisia. The production of *Cucurbita* is based on local accessions and landraces with the exception of zucchini where imported F1 hybrids are cultivated in greenhouses for immature fruit production.

Pumpkin collections at the Regional Research Centre on Horticulture and Organic Agriculture (CRRHAB, Tunisia) were initiated in 2016 and some accessions were collected from different regions of Tunisia (Figure 1). Several studies were initiated to characterize those landraces. Chikh-Rouhou et al. (2019) showed that these landraces correspond to three species: *C. maxima*, *C. pepo*, and *C. moschata* with a predominance of *C. maxima*. Significant differences were found for all the phenotypic traits evaluated revealing a great diversity among the landraces and among the 3 species of *Cucurbita* especially in fruit shape, size (Figure 1, Table 1), peduncle (angled or cylindrical) (Figure 2), leaves (rounded shallow lobes or lobes acute or obtuse; with or without whitish blotches) (Figure 3A), and growth habit (bushy, intermediate or prostrate) (Figure 3B).

The cultivation of *C. maxima* (winter squash) is based on local open-pollinated varieties which are maintained by farmers, produced for self-consumption and sold at local markets. Similarly, the production of *C. moschata* is based on local varieties for home consumption or sale at local markets. *C. pepo* (summer squash) is produced in greenhouses and open fields, and F1 hybrids are cultivated in greenhouses for zucchini production. Squash seeds have been used as a snack in Tunisia like in other Mediterranean countries.

Winter squash, summer squash, and pumpkin populations of Centre-East Tunisia showed high variability for seed size, color, and weight (Chikh-Rouhou et al. 2019). However, very few studies have been initiated regarding the characterization and utilization of Tunisian local germplasm. Hamdi et al. (2017) characterized 15 landraces of *C. maxima* using either morphological or biochemical traits and Hamdi et al. (2020) evaluated the population structure of a Tunisian and Italian *C. maxima* germplasm collection by combining morphological and molecular markers, observing a large qualitative variability according to fruit-related traits and effective discrimination of all the accessions. Also, Enneb et al. (2020) investigated the biochemical and nutritional properties of *C. moschata* extracts from pulp, fibers, and seeds, showing the great diversity of these landraces collected from arid lands of Medenine south Tunisia in regards to the biochemical traits evaluated.

In order to develop new varieties of pumpkin for seed production, selection studies are being conducted in the CRRHAB since 2018. In these studies, different seed sources were collected and are under evaluation. Varieties of naked-seed pumpkin (characterized by having a thin membranous seed coat, which makes the entire seed edible and easily pressed to extract the prized culinary oil) will be introduced and research will be conducted with this type of variety. Naked pumpkin seeds are a popular ingredient in many snacks, breads, breakfast cereals, soups, and other edible goods (Meru and Fu, 2021, Baxter et al. 2012). Pumpkin seeds are rich in oil (50% w/w), protein (35%), unsaturated fatty acids (86%) (Meru et al. 2018), and antioxidants that have many health benefits. Oil-seed pumpkins can be purchased by the bottle for culinary/condiment use or as capsules in health food stores (Stevenson et al. 2007).

Biochemical analysis of pumpkin landraces (flesh and seeds) is ongoing in collaboration with INRAT (Tunisia). On the other hand, screening for resistance to cucurbit powdery mildew (CPM) and downy mildew (*Pseudoperonospora cubensis*) is ongoing at CRRHAB to select the best accessions for further breeding programs. The first results are promising and some potential landraces resistant to CPM and with high yield are being identified (Chikh-Rouhou et al. in preparation). Pumpkin diversity and associated microbiota are being also determined (Aydi-Ben-Abdallah et al. in press). Indeed, comparative studies of plant microbiomes with resistant and tolerant phenotypes to different diseases in pumpkin accessions are ongoing at CRRHAB. Assessments for resistance to various soilborne pathogenic fungi using these accessions are also ongoing in collaboration with the Department of Plant Science of CITA (Spain).

In Tunisia, the most frequent use of pumpkins is the inter-specific F1 hybrids of *C. moschata* x *C. maxima* as rootstocks to melon and watermelon grafting, all of which are imported. Here we emphasize the utilization of our local landraces which might open the potential to create local rootstocks and save hard currency paid for importing seeds.

We emphasize here the need to evaluate local pumpkin diversity for more efficient management and utilization of landraces for sustainable conservation and valorization of the collected accessions. Indeed, phenotypic and molecular studies are necessary for more accessions of regional or national collections to determine the genetic diversity and structure of the local varieties, populations, landraces, hybrids, introduced accessions, and wild species.

Acknowledgements

Research laboratory LR21AGR03-Production and Protection for a Sustainable Horticulture, funded by the Ministry of Higher Education and Scientific Research of Tunisia. PID2020-116055RB-C22 I+D+I project funded by MCIN/AEI/10.13039/501100011003 and the A11-20R project funded by the Aragon Government.

Literature Cited

Aydi-Ben-Abdallah, R., H. Chikh-Rouhou, H. Jabnoun-Khiareddine and M. Daami-Remadi. 2023. Pumpkin (*Cucurbita* spp.) diversity and their associated microbiota. Advances in Horticultural Science (in press).

Baxter, G. G., K. Murphy, and A. Paech. 2012. The potential to produce pumpkin seed for processing in north east Victoria. Rural Industries Research and Development Corporation. RIRDC Publication No. 11/145.

Bisognin, D.A. 2002. Origin and evolution of cultivated cucurbits. Ciencia Rural 32:715-723.

Caili, F., S. Huan and L.A. Quanhong 2006. A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods for Human Nutrition 61: 70-77.Castetter E.F. 1930. Species crosses in the genus *Cucurbita*. American Journal of Botany 17 (1): 41-57.

Chikh-Rouhou, H., I. Fhima, D. Khechine and R. Sta-Baba. 2019. Diversity among pumpkins landraces (*Cucurbita* spp.) grown in Tunisia using fruit and seed quantitative traits In: Direk H (eds.), Proceedings Book of the 6th International Conference on Sustainable Agriculture and Environment (ICSAE). Konya-Turkey, 3-5 October 2019. Proceedings ICSAE: 578-581. ISBN: 978-605-184-194-6.

Enneb, S., S. Drine, M. Bagues, T. Triki, F. Boussora, F. Guasmi, K. Nagaz and A. Ferchichi. 2020.

Phytochemical profiles and nutritional composition of squash (*Cucurbita moschata* D.) from Tunisia. South African Journal of Botany 130: 165-171.

Gbemenou, U.H., V. Ezin and A. Ahanchede. 2022. Current state of knowledge on the potential and production of *Cucurbita moschata* (pumpkin) in Africa: A review. African Journal of Plant Science 16(1): 8-21.

Hamdi, K., J. Ben-Amor, K. Mokrani, N. Mezghanni and N. Tarchoun. 2017. Assessment of the genetic diversity of some local squash (*Cucurbita maxima* Duchesne) populations revealed by agro-morphological and chemical traits. Journal of New Sciences, Agriculture and Biotechnology 42(5): 2306-2317.

Hamdi, K., D. Palma, P. Angelini, N. Acciarri, N. Tarchoun and S. Sestili. 2020. *Cucurbita maxima* Duch. population analysis: relationship between Tunisian and Italian germplasm. Journal of Horticultural Science and Biotechnology 95(4): 496-505.

Hazra, P., A.K. Mandal, A.K. Dutta and H.H. Ram. 2007. Breeding Pumpkin for fruit yield and other characters. International J of Plant Breeding 1(1): 51-64.

Khoury, C.K., D. Carver, H.R. Kates, H.A. Achicanoy, M. van Zonneveld, E. Thomas, C. Heinitz, R. Jarret, J.A. Labate, K. Reitsma, G.P. Nabhan and S.L. Greene. 2019. Distributions, conservation status, and abiotic stress tolerance potential of wild cucurbits (*Cucurbita* L.). Plants, People, Planet Volume 2, Issue 3. https://doi.org/10.1002/ppp3.10085.

Meru, G., and Fu, Y. 2021. Yield and horticultural performance of naked-seed pumpkin in South Florida. HS1323. Gainesville: University of Florida Institute of Food and Agricultural Sciences.

Meru, G., Y. Fu, D. Leyva, P. Sarnoski, and Y. Yagiz. 2018. Phenotypic relationships among oil, protein, fatty acid composition and seed size traits in *Cucurbita pepo*. Scientia Horticulturae 233: 47-53.

Paris, H.S. 2018. Consumer-oriented exploitation and conservation of genetic resources of pumpkins and squash, *Cucurbita.* Israel Journal of Plant Science http://dx.doi.org/10.1163/22238980-00001036

Roberts, T. 2006. The many uses of pumpkins. Journal of Agriculture and Food Chemistry 49: 1253-1259

Stevenson, D.G., F. J. Eller, L. Wang, J. L. Jane, T. Wang, and G. E. Inglett. 2007. Oil and tocopherol content and composition of pumpkin seed oil in 12 cultivars. Journal of Agricultural and Food Chemistry 55: 4005-4013.

Tlili, I., H. Chikh-Rouhou, R. Ilahy, E. Jedidi, R. Bouhlel, L. Romdhane, S. Ghannem, MS. Lenucci, MW. Siddiqui, T. R'him, C. Hdider. 2020. Pumpkins. In: Nayik G.A., Gull A. (Eds.) Antioxidants in vegetables and nuts - properties and health benefits. pp 105-126. Springer, Singapore.

Whitaker, T.W and R.W. Robinson. 1986. Squash breeding. In: Bassett, M.J. (ed.), Breeding vegetable crops, pp. 209-242.

Figure 1. Diversity of pumpkin (*Cucurbita* spp.) landraces collected from local farmers and evaluated at the Research Centre on Horticulture and Organic Agriculture (CRRHAB), Tunisia.

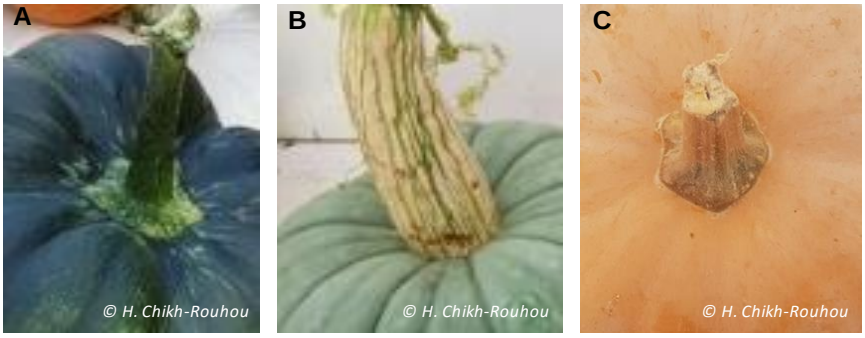

Figure 2. Variation in peduncles of *Cucurbita* landraces in Tunisia. (A) smoothly angled and expanded or flared at fruit attachment (*C. moschata*). (B) spongy, cylindrical, corky (*C. maxima*). (C) angled with little or no expansion at fruit attachment (*C. pepo*).

Figure 3. Leaf type and plant growth habit among *Cucurbita* species in the CRRHAB collection. A: Leaves (rounded shallow lobes or lobes acute or obtuse; with or without whitish blotches), B: Plant growth habit (a) bushy, (b) intermediate, (c) prostrate. (Photo H. Chikh-Rouhou)

Table 1. Range of values of phenotypic traits of landraces of three species of *Cucurbita* in the collection at the Research Centre on Horticulture and Organic Agriculture (CRRHAB), Tunisia.

Number of accessions	Species	Fruit weight range (kg)	Flesh thickness range (cm)	Seeds number range
10	*C. moschata*	1.4 – 17.1	1.5 – 7.3	81 – 774
15	*C. maxima*	4.8 – 25.3	3.0 – 13.1	90 – 524
5	*C. pepo*	0.5 – 4.2	1.5 – 6.5	31 – 435