

# Implications of the introgression between *Citrullus colocynthis* and *C. lanatus* characters in the taxonomy, evolutionary dynamics and breeding of watermelon

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## Summary

**Implications of the introgression between *Citrullus colocynthis* and *C. lanatus* characters in the taxonomy, evolutionary dynamics and breeding of watermelon**

*Citrullus colocynthis* is a wild species counted among the ancestors of the cultivated watermelon, *C. lanatus*. Cross-compatibility between these species through both natural and artificial hybridization has been widely reported. Watermelon landraces were collected from the northeast of Brazil and studies showed evidence of introgression from *C. colocynthis* to *C. lanatus*, although *C. colocynthis* is not distributed in Brazil. This paper discusses the implications of this introgression in the taxonomy, evolution dynamics and the improvement of watermelon.

**Key words:** Brazil, *Citrullus colocynthis*, *Citrullus lanatus*, evolution, introgression, watermelon

## Résumé

**Conséquences de l'introgression entre des caractères de *Citrullus colocynthis* et *C. lanatus* sur la taxonomie, la dynamique de l'évolution et l'amélioration de la pastèque**

*Citrullus colocynthis* est une espèce sauvage figurant parmi les ancêtres de la pastèque cultivée, *C. lanatus*. La compatibilité entre ces espèces par hybridation naturelle ou artificielle a été largement décrite. On a récolté des variétés locales de pastèque dans le nord-est du Brésil et démontré l'introgression de *C. colocynthis* dans *C. lanatus*, bien que *C. colocynthis* ne soit pas présent au Brésil. La présente étude examine les conséquences de cette introgression sur la taxonomie, la dynamique de l'évolution et l'amélioration de la pastèque.

## Resumen

**Implicaciones de la introgresión entre caracteres de *Citrullus colocynthis* y *C. lanatus* en la taxonomía, la dinámica evolutiva y la genética de la sandía**

*Citrullus colocynthis* es una especie silvestre considerada ancestro de la sandía cultivada, *C. lanatus*. La compatibilidad recíproca entre estas especies por hibridación tanto natural como artificial se ha señalado frecuentemente. Estudios realizados con variedades nativas de sandía recolectadas en el nordeste del Brasil revelaron la introgresión de *C. colocynthis* a *C. lanatus*, a pesar de que *C. colocynthis* no se ha extendido en Brasil. Este trabajo analiza las implicaciones de esta introgresión en la taxonomía, la dinámica evolutiva y la mejora genética de la sandía.

## Introduction

The *Citrullus* genus includes three species, of which watermelon (*Citrullus lanatus*) is the cultivated species. Romão (1995) reports on its broad morphological intraspecific variability; it also has low isozymic variability. Both morphologic and isozymic variability have been found in *C. lanatus* in several parts of the world where it has undergone introgressive hybridization with the wild species *C. colocynthis*. The resulting species has been classified as *C. lanatus* var. *citroides*. This paper reports on the occurrence of this variety in Brazil and discusses its implications for the evolution and the genetic improvement (breeding) of the species.

## Taxonomy of the *Citrullus* genus

The genus *Citrullus* (Cucurbitaceae) consists of three diploid species ( $2n=22$ ): (i) *C. lanatus* (Thunberg) Matsumura and Nakai, including the cultivated watermelon widely grown in several parts of the world, (ii) *C. lanatus* var. *citroides*, a wild form found in South Africa and also cultivated in other parts of the world mainly for feeding animals, and (iii) *C. colocynthis* (L) Schrad, found in the north

and southwest areas of Africa and Asia, which can be divided into two different races, one found on the Mediterranean coast and in Israel, the other found in the deserts of Negev and Sinai, and *C. ecirrosus*, which is endemic to the Namibian desert (Jeffrey 1975; Zamir *et al.* 1984).

*Citrullus lanatus* (Thunb.) Matsumura and Nakai is a cultivated species, originating in south or maybe central Africa; Whitaker and Davis (1962) also describe the existence of a secondary diversification centre in India. The species is characterized by large green leaves with three to five deep lobes on the edges, or more rarely none, medium-sized monoic flowers with short pedicels, medium to large fruit with smooth skin and flesh with a high water content, and oval to oblong seeds of a white or brown colour.

According to Fursa (1981), the cultivated species *C. lanatus* includes three subspecies: (i) *lanatus*, (ii) *vulgaris* which has two varieties, var. *vulgaris* and var. *cordophanus*, and (iii) *mucocospermus*. An ecogeographic classification of cultivated watermelons has no taxonomic purpose, however, with the aid of collections the influence of ecologi-



cal conditions, as well as of controlled crossing, on the development of different types of varieties could be shown. Based on the regions cultivating the variety, the leaf morphology and anatomic structure, physiological indexes (water retention capacity) of leaves and the sexual type of the plants, varieties have been grouped into three types: Russian, Asian and Occidental.

Maheshwari (1978) recognizes several watermelon varieties cultivated in different parts of the world, e.g. India, Pakistan, Malaysia, Polynesia, Japan, China, Iraq, Europe, Africa, and South and Central America. Among other characters, such varieties differ in size, shape and colour of fruit skin, colour of flesh (red, pink, white and yellow), and the colour and size of seeds. The author is aware of 13 varieties of *C. lanatus*: var. *lanatus* a wild watermelon native to southern Africa; var. *viridis* a 'giant' watermelon from Iraq and cultivar 'Black Tom Watson'; var. *albidus* in the *nigro-seminius* and *albidus* forms bred in the central areas of Iran; var. *variegatus*; var. *rotundus*; var. *pulcherrimus*; var. *shami*; var. *oblongus* whose common name is 'Fairfax'; var. *virgatus*; var. *pumilus* which is called 'New Hampshire'; var. *caffè* a sweet cultivated watermelon and var. *citroides*, whose common names are, respectively, 'citron melon' and 'preserving melon'.

According to Anghel (1969), *C. colocynthis* (L.) Schrad originates from North Africa and south Asia. Mohr (1988) has described it as a perennial species differing from *C. lanatus*, mainly in the size of the plant organs. Its leaves are small, with grey narrow hairy lobes, the flowers are small and monoecious and the seeds small and brown. The fruits are small, 15-20 cm wide in diameter, and spongy with a thin rind. When ripe they are a green to light-green or light-yellow colour, with white or compact flesh with a bitter taste. This species also possesses medicinal properties (Koshoo 1955). Smith and Cooley (1973) described the wild watermelon as having fruit of 15-20 cm in diameter, which produce from 600 to 1000 seeds per fruit. The pericarp is white with a low sugar content and the seeds are green.

Whitaker (1933), Shimotsuma (1960) and Anghel (1969) point to evidence that *C. colocynthis* is the ancestor of *C. lanatus*, based on cytogenetical investigations, intercrossing compatibility and dissemination in Africa and Asia.

### Morphological characters involved in domestication

The fruit of the wild species are characterized by white flesh and an extremely bitter flavour. This bitter taste is caused by a high concentration of a substance called Cucurbitacine E. glycoside (Herrington *et al.* 1986) or colocynthine (Mohr 1988) and is controlled by a (*Bi*) gene which is dominant over the non-bitter character (Robinson *et al.* 1976; Navot *et al.* 1990). The bitter taste is also present in wild species of other Cucurbitaceae (Joubert 1980).

The red colour of the flesh is influenced by a recessive (red) gene, but according to Navot *et al.* (1990), the colour inheritance of the flesh is more complex in character and involves the epistatic effect.

These characters have been the two most important ones in the domestication process as there is strong pressure for red flesh and a non-bitter taste. According to Navot *et al.* (1990), selection for the rare mutants, which have eliminated the bitterness and added red colour, has been responsible for the isozymic monomorphism found in cultivated watermelon accessions.

### Hybridization occurrence between *C. lanatus* and *C. colocynthis*

Natural hybrids between *C. lanatus* and *C. colocynthis* have already been found. Singh (1978) found one of these hybrids in India, where both species are well represented in the semi-arid regions of Rajasthan. *Citrullus lanatus*, called locally 'Matiro', *C. colocynthis* called 'Tumba' and other intermediate forms called 'Khar' or 'Tatumba' have been found growing close to each other. Morphological and cytological observations reinforce the close relationship between both species. Maheshwari (1978) also described a variety called 'Neri' as an almost intermediary form between *C. colocynthis* and *C. lanatus*. In Arizona, Fulks *et al.* (1979) found natural hybrids growing between *C. colocynthis* and *C. lanatus* cv. Citron, which had white flesh, bigger fruit and no characteristic bitter taste. Furthermore, Shimotsuma (1963) obtained artificial hybrids. Natural hybrids have also been reported in Australia (Herrington *et al.* 1986) and in Texas, USA where they invade plants growing on sandy soils such as cotton and sorghum. On some infested cotton fields they reduce production from 20 to 35% and interfere in mechanical harvesting. Similar problems occur with peanut cultivation (Smith and Cooley 1973).

Hybridization between the species has produced a specific classification for plants with intermediary characters, the nomenclature *C. lanatus* var. *citroides* being used. Based on isozyme data, Navot and Zamir (1986) consider this South African variety to be the wild progenitor of the cultivated watermelon. Based on immunochemical analyses, Fursa and Gavrilyuk (1990) support the hypothesis that *Citrullus lanatus* originated from *cordophanus*, the semi-cultivated variety found in Sudan.

### *Citrullus* germplasm in Brazil

Plant populations with the characteristics of *C. lanatus* var. *citroides* have been found in the northeast of Brazil and are known as fodder watermelon. In order to obtain germplasm to develop watermelon cultivars resistant to disease and to the risk of genetic erosion, CPATSA, the agricultural research centre for the semi-arid tropics attached to EMBRAPA, collected fodder watermelon.

Collecting was carried out on the land of farmers in different locations in the northeast region of Brazil, including the municipality of Pastos Bons in Maranhão state, the Irecê microregion in Bahia state, the Teresina area in Piauí state, the Chapada Diamantina plateau in Bahia state in the municipality of Jacobina, and from Tacaimbó in Pernambuco state (Ramos and Queiroz 1992; Queiroz 1993). The collected germplasm presented a wide morphologic variability



(Romão 1995; Ferreira 1996). Genes have been found resistant to the main diseases, such as viruses, powdery mildew and gummy stem blight, which attack the cultures in irrigated areas (Araújo and Souza 1988; Souza *et al.* 1988; Araújo *et al.* 1989; Dias *et al.* 1989; Dias 1993). According to Romão (1995) this germplasm was brought to Brazil by African slaves, therefore, watermelon has been cultivated in several parts of Brazil since the colonization period. Watermelon was introduced into North America by European colonizers in 1629 and grown as an economic crop (Mohr 1988). According to Costa and Pinto (1977) American immigrants brought watermelon to São Paulo in Brazil.

### Occurrence of *C. lanatus* var. *citroides* in Brazil

Fodder watermelons, originally found in Ouricuri, Pernambuco State, have been identified as a source of resistance against powdery mildew (Araújo *et al.* 1989) and tolerance of WMV-1 (papaya mosaic virus or watermelon stirps according to a more recent classification). According to reports by Sitterly (1972) and Ávila (1984) this is a disease against which there has been no resistance. This has resulted in a great deal of wastage, as all the cultivars used in Brazil are susceptible to this as well as other diseases. Yet

little work has been done on developing resistant forms of these cultivars in Brazil to date as the American types are well accepted. According to Araújo (1989), this has discouraged making improvements to this vegetable.

Cytogenetic analysis shows that fodder watermelon is similar in chromosome number ( $2n=22$ ) to the watermelon cultivars. Pollen grains, when observed under the electronic scanning microscope, show a similar structure between watermelon cultivars and fodder watermelon. The latter, however, showed heteromorphism, which is likely to indicate a possible hybrid origin. Electrophoretic divergence has been found for general proteins (Fig. 1) when compared with the cultivated forms.

A study of crossing compatibility with 10 cultivated genotypes has revealed distinct behaviour for each cultivar. Fruit with feasible seeds has been obtained from all crossings. Hybrids show a quite variable feasibility of seed ranging from 3 to 74%, while in general pollen grain feasibility was above 50%, except for one case where feasibility was estimated to be 33%. These reductions in seed as well as pollen grain feasibility are barriers to intercrossing between fodder watermelon and the cultivated genotypes.

The studies show the presence of *C. colocynthis* at the origin of these accessions, although there are no reports of this species existing in Brazil. It is possible that character introgression may have taken place in localities where fodder watermelon can be currently found, and that *C. colocynthis* was later eliminated through human selection. However, it is more likely that it was introduced in a form close to the fodder watermelon and has been kept for all these years for use as animal feed. Fodder watermelon shows a stable genotype, presenting partial hybridization barriers with cultivated genotypes.

The effect of human selection constitutes another crossing barrier between them, a consequence of which is the isolation of the *C. colocynthis* alleles borne by fodder watermelon, which facilitates its speciation process. In fact, farmers in northeast Brazil feed fodder watermelon to their animals during the dry season as they have large fruit (sometimes more than 15 kg).

Nevertheless, the formation of a seed bank in the soil, with the aid of the cultivated watermelon's seeds (*C. lanatus* var. *citroides*) (Romão 1995), as well as the possibility of obtaining fertile and vigorous hybrids, allows the genus flow between these taxa and increases the variability in watermelon cultures. Furthermore, the use of white-fleshed watermelon as animal feed prevents these sub-spontaneous genotypes from becoming extinct. At a commercial watermelon plantation in Australia, Herrington *et al.* (1986) verified the introgression of bitterness due to the genus flow of non-bitter spontaneous white-fleshed watermelon. Intercrossing progeny from both has resulted in 67 phenotypes of different fruit.

In spite of the fact that white flesh character is associated with bitterness (Navot *et al.* 1990), there are reports of landraces where these characteristics can be found dissociated both in *Citrullus lanatus* and in *C. colocynthis*, which is a

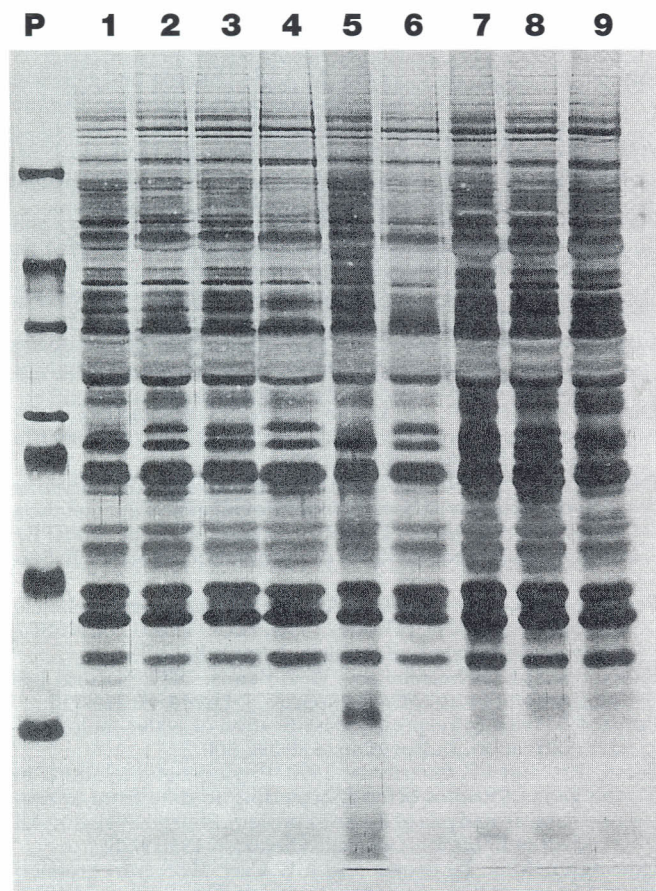


Fig. 1. Patterns of seed proteins: (1) watermelon cv. Sunshade x Fodder watermelon (HW), (2) cv. Sunshade, (3) cv. Omaru Yamato x HW, (4) cv. Omaro Yamato, (5) cv. Charleston Gray x HW, (6) cv. Charleston grey, (7) HW, (8) *Citrullus colocynthis* (Nigeria), (9) *Citrullus colocynthis* (Namibia).



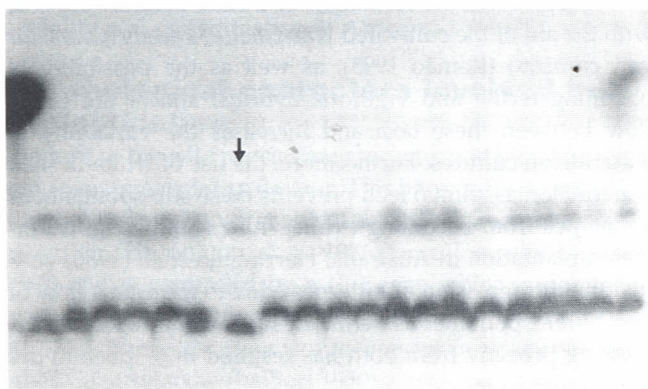
wild ancestral species of the cultivated form (Shimotsuma 1960, 1963; Maheshwari 1978). Thus, among landraces of watermelons cultivated by small farmers in the northeast region it is not difficult to find small-sized white-fleshed fruits with a very sweet taste.

Such facts are extremely advantageous for breeding as interesting characters are present in genotypes which have a greater morphological proximity to the cultivated watermelon than wild species, which apart from having white flesh, usually possess undesirable fruit and plant characteristics (small fruit, large number of seeds, low sugar content). Moreover, it has been demonstrated that if we consider the seed germination time and height of seedling as parameters, the hybrids between the fodder watermelon and the cultivated genotypes appear vigorous five days after germination (Assis 1994). This suggests the presence of heterosis.

### Isozymatic polymorphism and evidence of introgression between cultivated and non-cultivated watermelons

Forty-five accessions from the Cucurbitaceae germplasm bank of CPATSA/EMBRAPA collected in three regions: Médio Sertão Maranhense (MA), Depressão Sertaneja (DS) and Região Central da Bahia (BA) in northeast Brazil, were characterized in starch gel electrophoresis, including six accessions of the non-cultivated fodder watermelon and one cultivar 'Crimson Sweet'. Using six enzymatic systems: acid phosphatase (ACP), esterase (EST), catalase (CAT), peroxidase (PER), phosphoglucomutase (PGI) and malic enzyme (ME), 13 loci were scored, 10 being polymorphic. Mean heterozygosity, mean number of alleles per locus and percentage of polymorphic loci suggest that watermelon populations show higher variability than the 'Crimson Sweet' cultivar, and are closer to the values of non-cultivated fodder watermelon.

Five of the 10 polymorphic loci showed divergent patterns between watermelon landrace accessions and fodder watermelon, suggesting the existence of two gene pools (Fig. 2). Accessions from the three regions, mainly BA and MA,



**Fig. 2.** Electrophoretic pattern to phosphoglucoisomerase (PGI) in watermelon (arrow: variety 'Crimson Sweet'; right arrow: fodder watermelon from Bahia; left arrow: fodder watermelon from Rio Grande do Norte, suggesting introgression).

showed introgression from the other gene pool. Therefore, the gene flow evidenced by the introgression of isozymatic alleles, as a result of allogamy and cross-compatibility between both gene pools, may be the main cause of isozymatic variability. There was less variability in the DS region, which had a larger proportion of common alleles than the other regions. This is a tendency of improved cultivars, probably because collecting in this region was more selective. However, the morphological analysis showed this region to be the most varied, therefore, there is no relationship between isozymatic and morphological variabilities.

### Availability of seed samples

The EMBRAPA Research Unit is responsible for germplasm exchange and small quantities of seed samples of fodder watermelon and local watermelon populations can be obtained by writing to the Genetic Resources and Biotechnology Center SAIN, Parque Rural, Av. W5 Norte, Brazil CEP 70770-900; CP 02372, Fax (061) 340-3624, cenargen@cenargen.embrapa.br, Brasília-DF, Brazil.

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