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Pre-breeding in the watermelon germplasm bank of the Northeast of Brazil

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Summary

The use of germplasm from gene banks is limited and there is great concern in the scientific community about this problem. Ounce in the management of a germplasm bank the accessions can be studied in any of its different phases, this approach has been followed at the watermelon germplasm bank as a strategy to increase the use of the accessions in breeding programmes to improve the watermelon crop. Therefore, during the seed multiplication and characterization in field trials, both plant and fruit characters were recorded. It was found genetic variability for several descriptors used. In a multivariate analysis it was found nine different groups, although one group comprised around 68% of the accessions. It is expected that molecular markers e.g. RAPD or AFLP, help in the establishment of a core collection for watermelon. In another experiment, some accessions were evaluated against PRSV-w, WMV-2 and ZYMV, using ELISA test. It was found potential resistant plants for the three viruses which were transplanted to get selfed and crossed seeds. The resulting progenies and segregating populations could be analyzed using molecular tools. Past experiments revealed agronomic traits (powdery mildew resistance, prolificacy and small fruits) which were introgressed into commercial backgrounds to obtain lines and hybrid combinations of different ploidy levels and fruit patterns. With this approach around 84% of the accessions of the watermelon gene bank were used.

Keywords: *Citrullus lanatus*, genetic resources, gene bank management

Introduction

The watermelon crop in Brazil covers an area of 70,000 hectares in different parts of the country and produces around 1,000,000 tons of fruits annually (Queiroz et al., 2000). As also mentioned by the authors, the cultivars available for the farmers are few and were not developed for the Brazilian conditions, with very few exceptions. As a result, the productivity is low and the crop needs sprays against pests and diseases.

The watermelon has a great genetic variability in the traditional agriculture in the Northeast of Brazil. Several expeditions were performed and the samples collected gave rise to a watermelon germplasm bank (Queiroz et al., 1999) which comprises more than 500 accessions.

The use of accessions from a gene bank in breeding programmes has been of great concern in the scientific community (Brown et al., 1989), and Marshall (1989) analyzes the question in depth. Marshall (1989) discusses an appropriate measure for the

use of an accession of a gene bank. Peeters and Williams (1984) concluded that the number of independent requests for accessions is probably the simplest and the most appropriate measure. However, apart from the measure of usage there are other factors which have great influence in the germplasm use, e.g., lack of information in germplasm collections, lack of tuning between curator and breeders, legal constraints, low number of plant breeders and lack of pre-breeding among others (Marshall, 1989).

On the other hand, the management of a germplasm bank involves collecting, the multiplication, the characterization, the evaluation and the conservation, all focused in the use of the germplasm (Hawkes, 1982). Therefore, some of these phases were used to study the accessions of the watermelon germplasm bank, taking into account the points raised by Marshall (1989) regarding the use of accessions as it is described as follows.

Material and methods

The accessions were collected in the traditional agriculture in the Northeast of Brazil as seeds or fruits from the farmers fields or from open fairs. The seeds were stored in a cold chamber at 10°C and 40% of relative humidity (Queiroz et al., 1999).

The multiplication of the accessions was done under field conditions. During the multiplication trials plant and fruit characteristics were recorded. In a particular experiment, carried out in the year 2002, 48 accessions from Vitoria da Conquista, State of Bahia were planted in rows of fifteen plants for each accession. It was used self pollination for the majority of the plants, but some open pollinated plants were also harvested (half-sib progenies). The same experiment was also used in order to record disease resistance (grading scale: 1 = in the harvest, healthy plants with green leaves from the cotyledonary ones; 2 = defoliated plants with typical symptoms of powdery mildew and/or alternaria leaf blight; and 3 = other symptoms, including nutritional problems), fruit length and diameter, total soluble solids, flesh color and external color (Silveira and Queiroz, 2003).

In another experiment carried out in the year 2003, a set of 43 accessions from three different regions of the State of Bahia (Vitória da Conquista, Chapada Diamantina and Irece) was characterized using morphological descriptors (stem length, number of stems, yield per plant, fruit weight, length and diameter, flesh color in a scale from 1 (red) to 5 (white) and total soluble solids). The accessions were replicated in a randomized block. Univariate and multivariate analyses were performed (Cruz and Regazzi, 1994).

Finally, a set of nine accessions plus a check (cv. Crimson Sweet) were evaluated against papaya ring spot virus, strain watermelon (PRSV-w) and watermelon mosaic virus 2 (WMV-2). Another set of five treatments plus the same check were evaluated against zucchini yellow mosaic virus (ZYMV). A sample of each treatment was planted under green house conditions, using a subsample of eight plants to be inoculated with each virus. After ten days a second inoculation was done in the symptomless plants. A sample of 0.1g of leaf tissue of each plant was collected and analyzed using ELISA test (Almeida and Lima, 2000). Non inoculated plants (control) from the accessions were used. The plants which gave absorbancy values below twice the mean

absorbancy of non inoculated plants and not presented virus symptoms were regarded as resistant and were transplanted under field conditions in order to get seeds.

Results and discussion

Almost 50% of the evaluated accessions presented resistant plants to powdery mildew (*Sphaerotheca fuliginea*) and alternaria leaf blight (*Alternaria* sp.), but the percentage of resistant plants in each accession, ranged from 7 to 37%. Doing the morphological characterization of the fruits simultaneously to the seed multiplication, allowed to identify a reasonable genetic variability for fruit weight (0.6 to 7.6 kg), total soluble solids (2.0 to 10.2 ° Brix), fruit length (14.1 to 41.5 cm) and diameter (7.2 to 18.9 cm), external color (light to dark green, solid and strips of different widths) and flesh color (pink to red).

The morphological characterization in a replicated trial has also shown that the accessions collected in the State of Bahia presented genetic variation for plant and fruit characters, although the precision of the trial has been low, particularly for characters that are more influenced by the environment as yield and fruit weight (Table 1).

Table 1. Summary of the analysis of variance, average and amplitudes for some characters evaluated in 43 watermelon accessions collected in the State of Bahia, Petrolina-PE, 2003

Characters ¹	Mean Squares			C.V. (%)	Amplitude		
	Blocks (2d.f.) ¹	Accessions (42d.f.)	Error (84d.f.)		Mean	Lower Value	Upper Value
Stem length	4.047	3.265**	0.685	15.57	5.32	2.59	8.12
Number of stems	12.770	4.676*	2.842	19.05	8.84	3.67	13.67
Yield per plant	0.163	4.597*	2.383	28.64	5.38	1.53	10.27
Fruit weight	0.291	4.497**	0.915	28.30	3.38	1.20	11.00
Fruit length	0.454	50.040**	10.440	14.49	22.29	13.27	34.33
Fruit width	1.288	8.750**	1.782	8.88	15.02	10.70	23.63
Flesh color	0.195	1.055**	0.209	11.53	3.96	1.00	5.00
Total soluble solids	1.908	2.438**	0.387	9.69	6.41	3.73	11.13

¹d.f. – Degree of freedom; **, * Significant at 1% and 5% by F test, respectively.

A multivariate analysis showed two groups. One was made up by the check (Crimson Sweet) and the second was composed by all the other accessions. When the check was excluded from the analysis, nine groups were formed (Table 2). When analyzing the nine groups, the first cluster comprises accessions from the three regions, but the other clusters were formed by accessions of the same regions which, in turn, indicates a reasonable discrimination of the accessions. Molecular markers (Levi et al., 2000) could be used to analyze the same set of accessions in order to examine if the power

of discrimination increases, since they are not affected by the environment. They can also be used to analyze a representative sample of the accessions from the bank to establish a core collection (Brown, 1989) for long term conservation.

The absorbancy of the plants evaluated for PRSV-w, WMV-2 and ZYMV varied among the progenies in the accessions evaluated (Table 3). The non inoculated plants (control) also varied in the absorbancy values for the three viruses analyzed (Table 3).

Table 2. Similarity groups among 40 watermelon accessions according the Tocher's method based on the Mahalanobis distances. Petrolina-PE, 2003

Groups	Accessions
I	03 05 07 08 09 11 12 13 14 15 16 19 20 25 29 30 31 32 33 34 35 36 37 38 40 41 42
II	01 04 06
III	18 22 23 27
IV	24
V	21
VI	17
VII	2
VIII	28
IX	10

Table 3. Absorbancy values of the watermelon treatments evaluated against the virus PRSV-w, WMV-2 and ZYMV. UFC. Fortaleza-CE, 2003

Treatments	Absorbancy Amplitude		Treatments	Absorbancy Amplitude
	Virus PRSV-w	Virus WMV-2		Virus ZYMV
1	- ¹	0.409 – 3.063	1	0.360 – 3.075
2	0.123 – 2.136	0.189 – 2.084	2 (check)	0.243 – 3.166
3	0.122 – 0.624	0.178 – 0.765	3	0.239 – 3.163
4	0.127 – 0.155	-	4	0.205 – 3.515
5	-	0.116 – 0.329	5	0.247 – 2.365
6	-	0.120 – 0.166	6	0.192 – 2.212
7	0.114 – 2.997	-		
8	0.102 – 0.115	0.152 – 0.172		
9	0.104 – 0.116	0.156 – 0.248		
10 (check)	0.106 – 0.177	0.142 – 0.197		
Control	0.114 – 0.174	0.156 – 0.196		0.106 – 0.263

¹Not evaluated

The mean absorbancies for the control for the three viruses were 0.146 (PRSV-w), 0.175 (WMV-2) and 0.166 (ZYMV). It was found some plants with absorbancy values below the double mean for each virus (resistant plants), although some of them were discarded despite the low amount of virus, because they presented virus symptoms. It was selected 26 potential plants to be source of resistance to PRSV-w and 30 to WMV-2. For the virus ZYMV seven plants were selected.

It will be necessary to evaluate the progeny of the selected plants in order to identify homozygous sources for the three viruses, which, in turn, allows to obtain segregating populations for the study using molecular markers associated with resistance to those viruses (Danin-Poleg et al., 2000).

Considering past and recent experiments in the watermelon gene bank, several traits as powdery mildew resistance (Borges, 1996), gummy stem blight (Dias, 1993), virus (Oliveira et al., 2000), prolificacy and small fruits (Ferreira, 1996) were identified. In fact, as stated by Romão (1995) the majority of the genes described in the current watermelon literature have been identified in the traditional agriculture of the Northeast of Brazil. Also, tetraploid lines from the accessions have been developed (Souza et al., 1999).

Some traits have been transferred to commercial backgrounds and, then, inbred lines and hybrid combinations of different ploidy levels and fruit patterns, resistant to powdery mildew were developed (Queiroz et al., 2003).

Conclusions

With the approach described, the use of the accessions in the watermelon breeding programme has been around 84% which is superior to the use of several other crops according to the current literature. Therefore, this strategy can be applied to increase the use of accessions in gene banks of annual crops.

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