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Towards increasing diversity of local chili pepper (*Capsicum annuum* L.) gene pool in Tunisia: agronomic evaluation of a newly acquired gene collection

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Abstract - A gene collection of chili pepper entries (*Capsicum annuum* L.) was acquired from AVRDC (The World Vegetable Center, Taiwan). Twenty seven chili pepper entries were studied for genetic diversity during March-July 2012 in Tunisia. Six quantitative fruit characters (average fresh weight, diameter, length, pericarp thickness, total soluble solids and yield) were considered. Principal Component Analysis (PCA) was applied to establish the existing genetic diversity within the chili peppers' collection. The PCA revealed considerable variability among this collection: six clusters segregated based on two principal components accounting for 73.2% of total variance. Evidence was given on the large genetic diversity existing in these entries. Referring to additional fruit traits related to disease resistance and Tunisian consumer preferences, *e.g.* fruit color and fruit shape, a selection of several entries are proposed to further evaluation as new varieties and/or for potential use as parents in future breeding programs.

Keywords: *Capsicum annuum*, pepper assortment, variability, Tunisia.

Abbreviation list - Principal component analysis (PCA), principal components (PC), average fresh fruit weight (FFW), fruit length (FL), fruit diameter (FD), fruit pericarp thickness (PTh), total soluble solids (TSS), average yield (Yd), standard (Std.), minimum (Min.), maximum (Max.), coefficient of variance (CV) .

1. Introduction

Peppers (*Capsicum annuum* L.), particularly chili peppers, are economically important crops all over the world. Peppers are cultivated for several purposes: culinary use as vegetable, food ingredient or colorant (Loizzo et al, 2015; Materska 2014; Pugliese et al, 2013); cosmetics or medicinally and medically uses (Halikowski Smith 2015; Materska et al, 2015;Cordell and Araujo 1993; Palevitch and Craker 1995; Cichewicz and Thorpe 1996). In Tunisia, pepper cultivars are mainly represented by *C. annuum* species with a number of chili pepper landraces (Lahbib et al, 2013) cultivated throughout the country, in addition to a few *C. frutescens* cultivars (Zhani et al, 2015a, 2015b). However, degradation of domestic vegetable seed industry and massive introduction of foreign hybrid varieties seriously affected the local vegetable gene stocks. Indeed, most of cultivated pepper varieties are hybrids belonging to the *C .annuum* species (Zhani et al, 2015a). All of them, about 40 hybrids, are imported by local input suppliers. Whereas pepper cultivars, probably developed by spontaneous crossing and selection, are mostly maintained and multiplied by farmers for personal utilization. Hence, most of local pepper cultivars are actually composed of mixed genotypes populations*: e.g.* Baklouti Essahel, Rouge Long, Chaabani, M'sarreh, Fort de Korba, Baklouti Kairouan and Piment Sesseb (Ben Mansour-Gueddes et al, 2010). Great effort is consequently still needed to get homogenized genotypes. Since genetic resources are indispensable for selection and breeding programs, the present work was set up with consideration of the local seed market figure. In fact, in order to enhance pepper gene pool diversity in Tunisia, a collection of 26 chili pepper lines originating from AVRDC (The World Vegetable Center) were acquired. Adaptation and diversity assessment of these pepper lines was performed based on data from open field trials conducted under hot season cultivation environment, in comparison to one chili local pepper cultivar. Four morphologic fruit descriptors, *i.e.* fresh weight, length, diameter and wall thickness, as well as total soluble solids and fruit yield were assessed. Principal component analysis

(PCA) multivariate method was applied for the chili pepper collection's assessment. The main objective of this approach was to identify the existing genetic diversity within chili collection entries and to distinguish potentially suitable material for future utilization as new varieties or eventual genitors in future breeding programs.

2. Material and Methods

2.1. Experimental site

The experiment was conducted in Teboulba region located in central coast of Tunisia (latitude 3538'60.000"N, longitude 1058'0.120"E, altitude 17m) from April to July 2012. Teboulba is under a Mediterranean climate with daily high temperatures of 17-33°C and low temperatures of 9-24°C. Annual rainfall varies from 280 to 400mm.

2.2. Plant material and experimental design

Twenty six chili pepper entries (Table 1) were acquired from AVRDC gene bank. One local pepper variety, Baklouti, was also included in the present work as a local control. The experiment design was a randomized complete block with three replications. Each plot contained 20 plants. Plant spacing was 40 cm within and 100 cm between rows. Plants were watered with a drip irrigation system and they were cultivated using the standard horticultural practices in the region for open field pepper varieties.

Table 1. Chili pepper entries representing different pepper lines from AVRDC gene bank and one Tunisian local pepper variety

Original name	Country of origin
1. **Baklouti**	Tunisia
2. **VI041280 (PBC142)**	India
3. **AVPP0302**	Taiwan
4. **AVPP0303**	Taiwan
5. **AVPP9813**	Taiwan
6. **AVPP0304**	Taiwan
7. **AVPP0305**	Taiwan
8. **AVPP9905 (9955-15)**	Taiwan
9. **AVPP0306**	Taiwan
10. **AVPP0105**	Taiwan
11. **AVPP0307**	Taiwan
12. **VI041280-A (PBC142-A)**	India
13. **AVPP0903**	Taiwan
14. **AVPP0904**	Taiwan
15. **AVPP0905**	Taiwan
16. **AVPP0906**	Taiwan
17. **AVPP0907**	Taiwan
18. **AVPP0908**	Taiwan
19. **AVPP0909**	Taiwan
20. **AVPP9703**	Taiwan
21. **AVPP9704**	Taiwan
22. **AVPP9801**	Taiwan
23. **AVPP9806**	Taiwan
24. **AVPP9811**	Taiwan
25. **AVPP0606**	Taiwan
26. **AVPP0706**	Taiwan
27. **AVPP9805**	Taiwan

2.3. Fruit characterization

Fruit characterization was based on several fruit descriptors (average fresh fruit weight (FFW), fruit length (FL), fruit diameter (FD) and fruit pericarp thickness (PTh)), total soluble solids (TSS) and average yield (Yd), calculated respectively in the following units: g, mm, mm, mm, °Brix and kg/plant. Twenty pepper fruits for each entry were harvested at maturity stage and used for fruit characterization. Fruit yield was estimated from 30 plants for each entry using 10 randomly selected plants from each replication.

2.4. Statistical analysis

Multivariate PCA, using Varimax rotation method and Kaiser normalization, was applied to the data set corresponding to the chili pepper entries. Mean values were calculated for each entry based on 10 plants' average (yield parameter) or individual fruits (fruit descriptors and TSS) values. Statistical analyses were performed using the SPSS program, IBM Statisitcs 20.0 (IBM Corp. 2011).

3. Results

Significant differences among the collection of *C. annuum* chili peppers were shown for all the studied characters (Tables 2). Mean values, standard (Std.) Error, minimum (Min.) and maximum (Max.) values showed wide range of all parameters measured. In addition, coefficient of variance (CV) ranges from 24.3 (FL) to 69.3 % (FFW). These results are giving evidence on considerable genetic diversity occurrence within the pepper collection.

Table 2: Mean performance of 27 chili pepper entries as shown by mean values, standard (Std.) Error, minimum (Min.) and maximum (Max.) values, and coefficient of variance (CV).

	Mean	Std. Error	Min.	Max.	CV (%)	Sig.
FFW (g)	9.71	0.32	1.40	45.00	69.3	<0.0001
FL (mm)	97.34	1.14	47.41	166.00	24.3	<0.0001
FD (mm)	14.50	0.23	7.05	38.91	32.7	<0.0001
PTh (mm)	1.47	0.03	0.21	3.00	35.7	<0.0001
TSS (°Brix)	10.24	0.14	4.00	18.50	28.4	<0.0001
FYd (Kg/plant)	1.03	0.04	0.39	2.82	37.8	<0.0001

Aiming to identify divergent entries' groups, multivariate PCA was applied to the chili pepper data set. Multivariate PCA of chili pepper entries based on fruit descriptors (fresh weight, length, diameter and pericarp thickness), TSS and average yield, came out with two principal components (PC1 and PC2). Table 3 shows proportional share of each trait in these two PC. PC1 explained 46.41% of the total variance, and its loading was for three fruit descriptors: fresh weight, diameter and pericarp thickness. PC2 explained 26.78% of the total variance and it is accounted for by fruit length, TSS and yield. TSS is also negatively correlated with PC1. The two principal components accounted for 73.19% of the total variance.

Table 3. Eigenvector values for principal components using fruit descriptors and average yield of chili pepper collection.

Trait	PC1	PC2
Fruit fresh weight	**0.269**	0.095
Fruit length	-0.009	**0.445**
Fruit diameter	**0.282**	0.021
Fruit pericarp thickness	**0.303**	0.030
Total soluble solids	-0.391	**0.393**
Average yield	-0.196	**0.624**
Eigenvalue	2.784	1.607
Percentage of variance (%)	46.41	26.78
Cumulative variance (%)	46.41	73.19

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization Scores.

Clusters I and II (CI and CII) had high PC2 (Figure 1). In the opposite, CIV had low PC2.Entries in CI (20, 22, 23, 24 and 27) and CII (4, 8 and 25) had similar profiles regarding fruit length, TSS and yield

(PC2), but they differ at fruit fresh weight, fruit diameter and fruit pericarp thickness (PC1) with higher scores in CII.

CIV, containing entries number12, 13 and 17, had intermediate score of C1, but lowest score of C2. Accordingly, this cluster is characterized by small fruits and low yield: average parameters in CIV are FFW=2.69g, FD=9.80mm, PTh=1.03mm and Yd=0.67kg/plant. Entries in CIII clustered at the center of Figure 1 suggesting intermediate values of all parameters. It is the largest cluster including 14entries, referring to following trait averages: FFW=9.30g, FD=14.27mm, PTh=1.69mm, FL=98.96mm, TSS=8.24°Brix and Yd=0.97kg/plant.

Cluster CVI enclosed entry 26, while CV harbored local variety Baklouti only. They located at two opposite positions and had lowest and highest scores of PC1 respectively, but similar scores of PC2. It follows that the two entries had similar performance regarding TSS and yield but differ at fruit size and weight parameters where local Baklouti variety had higher scores, *i.e.* it has heavier and bigger fruits.

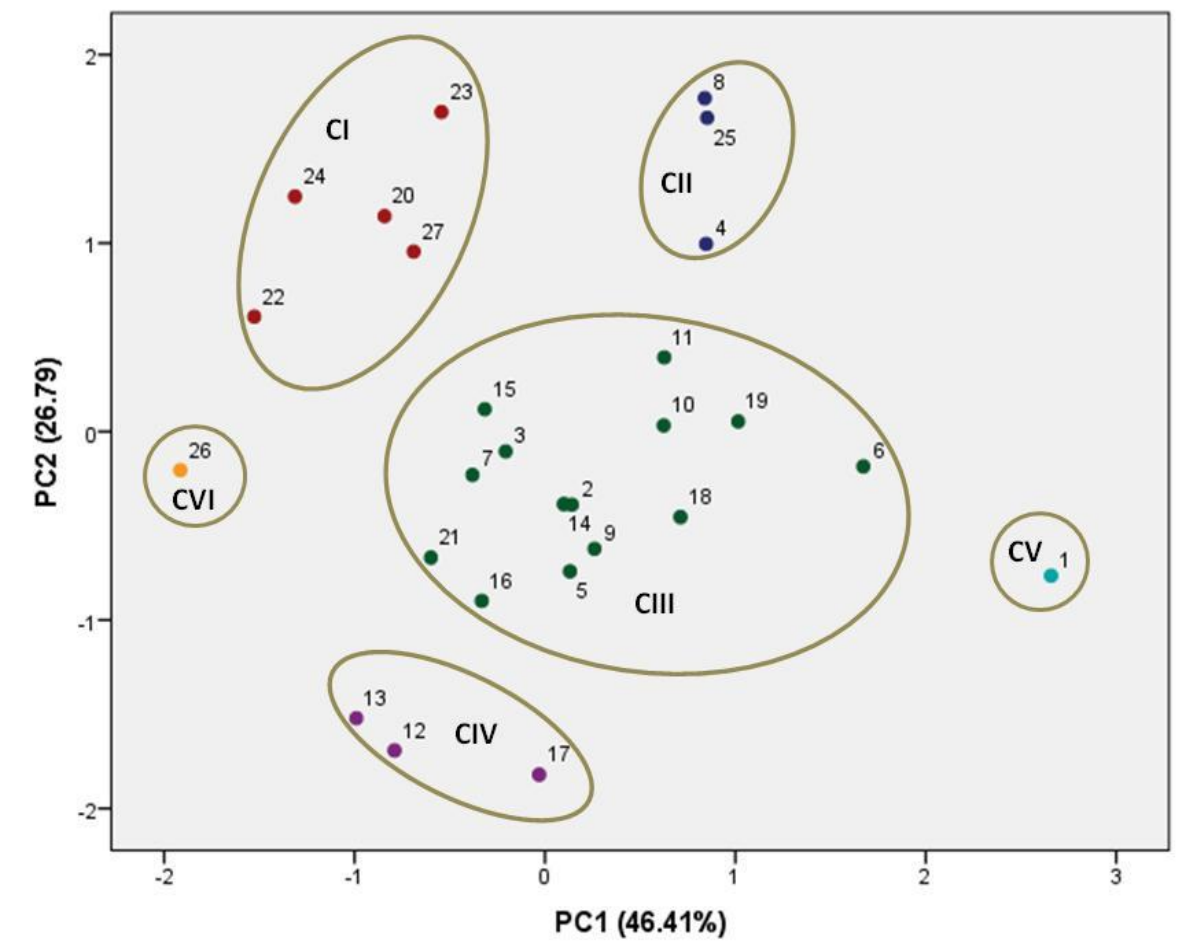

Figure 1: Plot of first and second principal components of fruit descriptors besides total soluble solids and average yield of 27 chili pepper entries at fruit maturity.

To sum up, cluster CIII was the largest one covering 14 entries characterized by intermediate values of all parameters. Whereas clusters CI and CII contained 5 and 3 entries, respectively, and performed the best for fresh fruit weight, fruit diameter and fruit pericarp thickness. Local variety Baklouti formed cluster CV by itself. Unsurprisingly, this variety is of potential interest to be used as parent in breeding program because of its phenotypic and horticultural characters.

Primarily, nine pepper entries were selected mainly for their fruit size adequate to local consumers' preferences (Table 4): entry 4 (AVPP0303, CII), entry, 6 (AVPP0113, CIII), entry 8 (AVPP9905, CII), entry 10 (AVPP0105, CIII), entry 11 (AVPP0307, CIII), entry 19 (AVPP0909, CIII), entry 23 (AVPP9806, CI), entry 25 (AVPP0606, CII) and entry 27 (AVPP9805, CI). All of these entries apart one (entry 19) had higher fruit yield than the local Baklouti variety (entry 1). Among these, illustration of five entries besides the local Baklouti pepper, showing fruit types suitable for local fresh market, is given in Figure 2. Entries 6 and 8 have both yellow fruit color that is not appreciated by Tunisian

consumers. Nonetheless, a particular effort will be given to these entries, primarily to entry 8 which recorded higher yield level. Entry 8 could be the first yellow chili pepper commercialized in local Tunisian fresh market.

Table 4. Fruit traits and yield performance of nine selected pepper entries and Baklouti local variety.

Entry	Weight (g)	Length (mm)	Width (mm)	Thickness (mm)	TSS (°Brix)	Yield (t/ha)
1	32.40	78.80	31.70	2.20	8.40	18.395
4	20.10	127.89	16.75	1.78	7.00	32.583
6	19.00	116.09	21.38	2.11	6.53	22.495
8	18.90	144.70	18.83	2.05	8.15	35.033
10	10.50	102.70	14.20	2.00	6.70	29.695
11	12.60	118.90	14.70	1.90	7.00	28.945
19	16.50	129.90	19.00	1.90	9.00	17.795
23	10.25	91.92	18.39	1.53	10.77	43.288
25	19.95	120.63	20.75	2.15	9.05	37.418
27	10.07	114.49	13.95	1.40	11.99	27.435

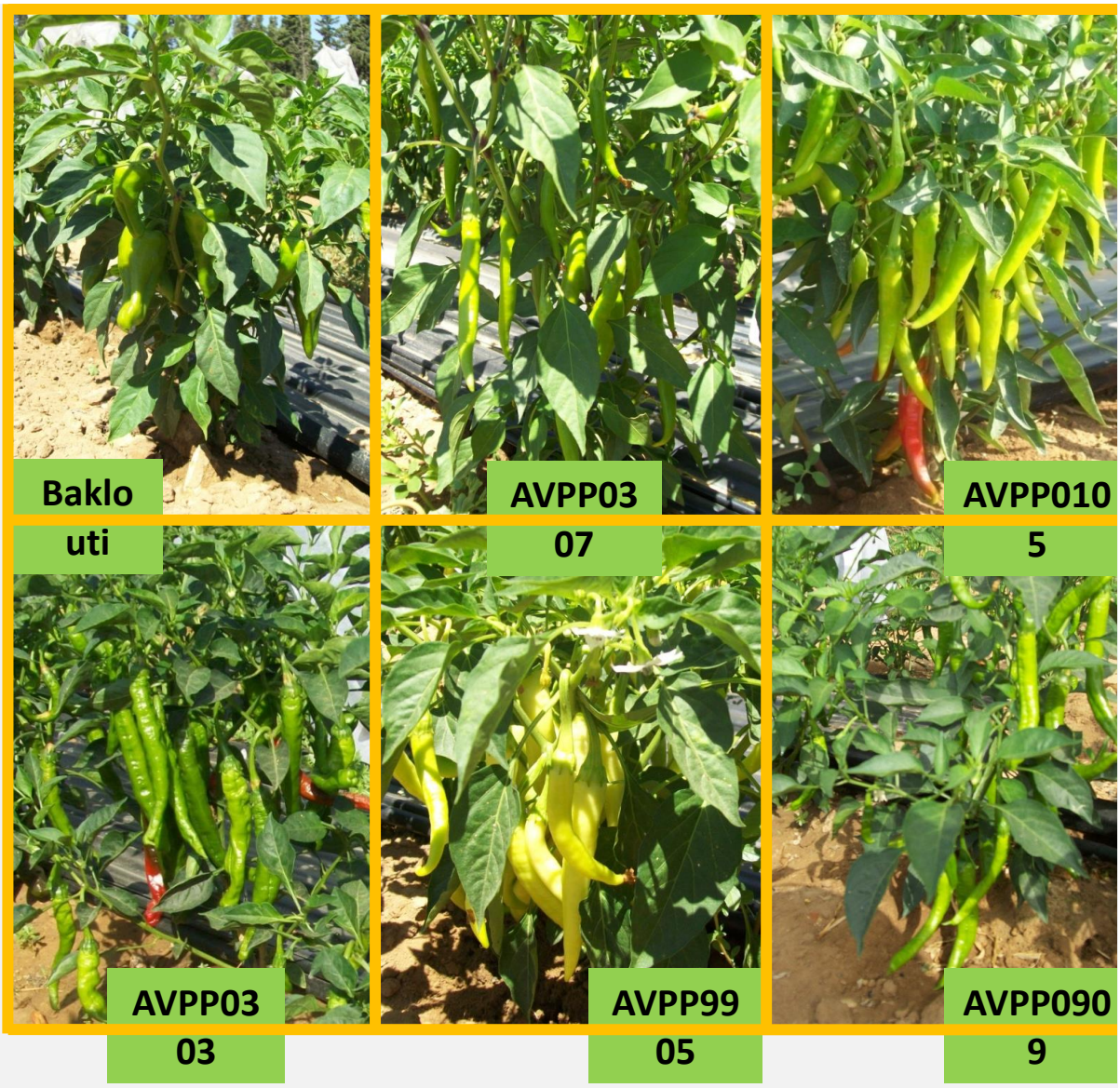

Figure 2: Pepper types and plant habit of five chili pepper entries and Baklouti local variety grown in open field conditions in Tunisia.

Remaining pepper entries had small fruits with fruit weight and fruit length ranging from 2 to 9 g and from 6 to 11 cm, respectively. In addition to their potential value as genitors in breeding programs, industrial use might be possible for some of these entries, especially those offering good yield levels. Nevertheless, this proposal hasn't been explored yet. For instance, entries 22 and 26 with high TSS levels (exceeding 14 °Brix) might represent an interesting material for industrial processing utilization.

4. Discussion

This work was carried out seeking to enrich the *C. annuum* gene pool in Tunisia using a collection of chili pepper lines acquired from AVRDC, which include entries from Taiwan and India.

Considering yield performance, average yield of pepper production in Tunisia was 16.8t/ha in 2012 (Observatoire National de l'Agriculture), while recorded yields during this work was from 12.7t/ha (entry 17) to 43.3t/ha (entry 23), and 6 of them yielded more than 30t/ha. Baklouti variety, used as a control, gives 18.4t/ha.

In Tunisia, pepper gene stock is mainly comprised of *C. annuum* besides *C. frutescens* species (Zhani et al, 2015a, 2015b; Lahbib et al, 2013). Most of Tunisian cultivars are maintained by farmers in a population structure, except very few cultivars which have been closely purified (*e.g.* Baklouti cultivar). Consequently, the majority of commercial pepper varieties are imported hybrids: more than 4.5t of pepper hybrid seeds have been imported in 2015. Importing highly productive varieties is valuable for pepper crop in Tunisia. However, it was reported that shipments have transported two harmful foreign diseases, *Verticillium* wilt and *Phytophthora* blight (M'Hamdi et al, 2009). These fungal diseases are causing severe losses both in field and greenhouse pepper production areas. Accordingly, producing pepper seeds locally will offer an alternative to enhance pepper production in Tunisia and prevent introduction of new diseases. Furthermore, useful information related to Tunisian consumer preferences (*e.g.* immature fruit color and fruit shape) should be considered during entries selection. From current collection of chili pepper, entries 23 and 25 had an additional interest; in fact they were shown to be resistant to *Phytophthora nicotianae* (Elbaz et al, 2015). In addition, based on laboratory assays performed in Taiwan (AVRDC), entry 23 was resistant to several viruses (CMV-Cucumber Mosaic Virus; CVMV-Chili Veinal Mottle Virus; PVY-Potato Virus Y and ToMV-Tomato mosaic virus).

Characters studied differentiated pepper entries into several groups from which superior hybrids could be achieved. Crosses that would be performed should be planned based on the genetic divergence and on the key agronomic traits. Parents of one cross should involve entries from distant clusters to increase variability and produce transgressive segregants allowing identification of material with high heterotic effects. Outputs from this work provide promising parent entries that should be considered as reservoir genes. They will definitely offer positive impact on the development of pepper seed sector in Tunisia. Interestingly, some of studied entries have already been released as open field varieties in several countries: entry 4 (Kazakhstan and Uzbekistan), entry 5 (Kazakhstan), entry 7 (Armenia), entry 10 (Kazakhstan and Mali) and entry 15 (Uzbekistan and Mali) (Lin et al, 2013). To conclude, the main objective of this work was reached since it came out with several pepper entries that possibly can be used either as new varieties or as parents in breeding programs.

5. Conclusion

This work was aiming to enrich the *C. annuum* gene pool in Tunisia using 26 chili pepper entries originating from Taiwan and India. The multivariate PCA came out with six clusters showing high variation among the pepper collection based on fruit descriptors, TSS and yield. Nine pepper entries, originating from three different PCA clusters, were selected for their fruit size adequate to local consumers' preferences. All of these entries, except one (entry 19), gave higher yield than the local control variety (Baklouti), and six of them yielded more than 30t/ha. Interestingly, considering disease resistance, entries 23 (43.3t/ha) and 25 (37.4t/ha) were shown to be resistant to *P. nicotianae* according to a previous work. Also, entry 23 is known as resistant to CMV, CVMV, PVY and ToMV. Moreover, further investigation on fruit quality will be performed to make a better selection of entries that will be involved in breeding work. Therefore, the main purpose of this work was reached and several pepper entries were shown to be potentially useful as new varieties or genitors to develop new hybrids. Finally, major outcome of this work is to contribute to build up a local pepper seed industry in Tunisia.

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