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Evaluation of *Triticum durum* Desf. germplasm for the improvement of local products

Stefania Marzario[1], Tania Gioia[2], Giuseppina Logozzo2, Pier Luigi Spagnoletti Zeuli[2]

[1] Dipartimento di Scienze, Università degli Studi della Basilicata, Potenza, Italy
[2] Scuola di Scienze Agrarie, Forestali, Alimentari ed Ambientali, Università degli Studi della Basilicata, Potenza, Italy

Abstract. The evaluation of '*ex situ*' germplasm collections allows us to use and conserve them more efficiently. A collection of 107 durum wheat (*Triticum durum* Desf.) accessions collected in Southern Italy from 1947 to 2009 was characterized using six morphological traits and 30 SSR markers. On the basis of the plant height, these accessions were classified into Pop 1 (plant height ≤70 cm), Pop 2 (70 cm <plant height <100 cm) and Pop 3 (plant height ≥100 cm). Significant differences were observed for morphological traits in these populations confirming the effects of the introduction of varieties with dwarfing genes. The 30 SSR markers identified 115 alleles, with an average of 3.83. The estimates of N_a, N_e, I and H_e for each population suggested a decrease in genetic diversity after the introduction of dwarfing genes in durum wheat germplasm. The level and the distribution of genetic diversity in the materials analysed in this study could be used as genetic criteria to protect these accessions in *ex situ* collections, to further investigate their characteristics and to use them both for breeding systems and for the improvement of typical products.

Keywords. *Triticum durum*, genetic resources, *ex-situ* conservation, morphological traits, SSR.

Evaluation des ressources génétiques de Triticum durum Desf. pour l'amélioration des produits locaux

Résumé. *L'évaluation des collections de ressources génétiques ex situ nous permet de les utiliser et de les conserver de manière plus efficace. Une collection de 107 accessions de blé dur (Triticum durum Desf.), collectées dans le sud de l'Italie de 1947 à 2009, a été caractérisée en s'appuyant sur six caractères morphologiques et 30 marqueurs SSR. Sur la base de la hauteur des plantes, ces accessions ont été classées en Pop 1 (hauteur de la plante ≤70 cm), Pop 2 (70 cm <hauteur de la plante <100 cm) et Pop 3 (hauteur de la plante ≥100 cm). Des différences significatives ont été observées pour les caractères morphologiques de ces populations confirmant les effets de l'introduction de variétés portant des gènes de nanisme. Les 30 marqueurs SSR ont identifié 115 allèles, avec une moyenne de 3,83. Les résultats obtenus pour Na, Ne, I et He dans chaque population ont suggéré une diminution de la diversité génétique après l'introduction des gènes de nanisme dans le matériel génétique de blé dur. Le niveau et la distribution de la diversité génétique dans le matériel analysé dans cette étude pourraient être utilisés comme critères génétiques pour protéger ces accessions dans des collections ex situ, pour mieux étudier leurs caractéristiques et les utiliser à la fois dans des systèmes d'amélioration génétique et pour l'amélioration des produits typiques.*

Mots-clés. *Triticum durum – Ressources génétiques – Conservation ex situ – Caractères morphologiques – SSR.*

I – Introduction

Durum wheat (*Triticum durum* Desf., 2n=4x=28; AABB genomes) is a tetraploid wheat species which is mainly used for human consumption. Over time in Italy plant breeding programs have introduced a number of varieties with always higher and more stable yield and improved grain quality that have continuously replaced the varieties previously locally grown. Genetic erosion of the available durum wheat germplasm has been prevented and a large number of accessions has been collected and preserved 'ex situ' for future breeding needs (Hagenblad *et al*., 2012). So far, only a small fraction of the huge worldwide collections of durum wheat have been characterized

and used. The analysis of genetic variation is a powerful tool to study germplasm resources and takes advantage by the development of a large number of molecular markers (Ganeva *et al*., 2010). Simple sequence repeats markers (SSR) or microsatellites, have been largely used to monitor the changes in genetic diversity in wheat germplasm (Landjeva *et al*., 2006; Mir *et al*., 2012).

In this study 30 SSR polymorphic markers are used (i) to assess the amount and the distribution of genetic diversity in a 'ex situ' collection of 107 accessions of durum wheat collected in the past 60 years in Southern Italy, and (ii) to evidence the genetic structure of the germplasm collected before and after the introduction of dwarf-gene varieties.

II – Materials and methods

1. Plant materials and morphological characterization

A germplasm collection of 107 durum wheat accessions that were collected in Southern Italy from 1947 to 2009 was used in the present study. Seed samples were kindly provided by the IPK genebank (Institute of Plant Genetics and Crop Plant Research), Gatersleben, Germany. Data were collected on morphological traits during the entire growing season in a field trial carried out at Azienda Agricola Sperimentale Dimostrativa (A.A.S.D.) Pantano in Pantano di Pignola, Potenza (Southern Italy) according to a randomized block design. Heading date, plant height, spike length, number of spikelet per spike, number of seeds per spike and weight of 1000 seeds were considered.

2. Genomic DNA extraction, PCR and SSR genotyping

For each accession genomic DNA was extracted from leaf tissues at the tillering stage using the automatic extractor ABI prism™ 6100 Nucleic Acid prep Station and the Trans-Prep protocol. Thirty SSR markers were selected on the base of their chromosome locations, T_{an} and degree of polymorphism. SSRs designation, chromosome location, primer sequences, T_{an} and the expected product size of the amplified loci were reported by Röder *et al*., (1998).

The PCR reaction was performed in a final volume of 50 µl composed of 10x PCR Buffer, 10 mM dNTP, 25mM $MgCl_2$, 10 µM forward primer, 10 µM reverse primer, 5 U AmpliTaq Gold DNA polymerase, 20 ng of genomic DNA and nuclease-free water. Amplification was performed by GeneAmp® PCR System 9700 as follows: 10 min at 95 °C; 1 min at 94 °C, 1 min at T_{an}, 1 min at 72 °C (35 cycles); and a final extension stage of 10 min at 72 °C. Following the PCRs, 3 µl of loading buffer 6x were added to 20 µl of each sample, then amplification products were analysed by electrophoresis in 2% agarose gels and visualised by ethidium bromide staining.

3. Statistical analysis

The germplasm collection was divided into three populations on the basis of the plant height: Pop 1 (plant height ≤70 cm), Pop 2 (70 cm <plant height <100 cm) and Pop 3 (plant height≥ 100 cm). Data were analysed by ANOVA using SAS 9.2 (TS2M3, SAS Institute Inc, NC, USA, 2002-2008) software package and means were compared by Duncan's multiple range test. Values were considered significant at $P < 0.005$.

For each SSR locus, the number of alleles detected, the gene diversity or unbiased expected heterozygosity (H_e; Nei 1978), and the polymorphic information content (PIC; Botstein *et al.*, 1980) were calculated using the program Power Marker 3.25 (Liu and Muse, 2005).

The genetic diversity in each population of durum wheat accessions was assessed using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012). Number of alleles (N_a), number of effective alleles (N_e), Shannon's diversity index (I) and gene diversity (H_e: Nei 1978) were computed.

III – Results and discussion

Table 1 summarises the mean values for morphological traits in each population of durum wheat accessions. The accessions of Pop 1, collected after the introduction of dwarf-gene varieties, are characterized by a lower significant value for heading date and spike length than Pop 2 and Pop 3; vice-versa for the other morphological traits Pop 1 showed higher significant values. These results are in accordance with the effects of the introduction of dwarf varieties with higher-yielding potential due to an increased harvest index and better lodging tolerance, especially under high fertilizer and water inputs. In these varieties the translocation of assimilates to the ear allowed a higher number of seeds per spike despite the lower number of spikelet per spike.

All SSR markers used in the present study showed polymorphic fragments among all the 107 durum wheat accessions analysed; in total SSRs revealed 115 alleles. The number of alleles per locus varied among markers, ranging from two (Xgwm165-4A, Xgwm169, Xgwm357, Xgwm408 and Xgwm415) to seven (Xgwm6), with an average of 3.83 (Table 2). As a measure of the discriminatory power of each microsatellite locus, the average PIC value was 0.47, ranging from 0.09 for Xgwm374 to 0.79 for Xgwm6 (Table 2). The average PIC value suggested that SSR employed resulted adequate and efficient, considering that a PIC value > 0.5 accounts for a highly informative marker, 0.5 > PIC > 0.25 for an informative marker, and PIC ≤ 0.25 for a slightly informative marker (Botstein *et al.*, 1980).

Table 1. Means of six morphological traits collected in 107 accessions of *Triticum durum* from Southern Italy.

Morphological Trait	Pop 1 (h≤70 cm) n=20	Pop 2 (70cm<h<100cm) n=63	Pop 3 (h≥100 cm) n=24
Heading date (d)	173.76 c	183.94 b	186.42 a
Plant height (cm)	58.63 c	89.60 b	110.74 a
Spike length (cm)	6.16 c	7.25 b	8.63 a
Number of spikelet per spike (n)	19.13 c	21.62 b	22.86 a
Number of seeds per spike (n)	50.98 a	46.81 b	46.92 b
Weight of 1000 seeds (g)	51.26 b	61.30 a	57.66 a

Duncan's multiple range test, P < 0.001.

The overall genetic diversity (H_e) was 0.529, indicating that the durum wheat accessions used in this study displayed a substantial level of genetic diversity. This value was lower compared with those reported in other studies in wheat using SSRs (Landjeva *et al.*, 2006; Mir *et al.*, 2012). These differences can be explained by considering the genetic background of genotypes studied, the number of markers used and the techniques applied to detect polymorphism.

In order to reveal the genetic structure of the germplasm collected before and after the introduction of dwarf-gene varieties, we estimated various standard statistics for each population of accessions of durum wheat (Table 3). Pop 1 included accessions with a lower value for the plant height and collected after the introduction of dwarf-gene varieties, showed lower genetic diversity for all statistics measured (Wilcoxon signed-rank test, $P < 0.001$) compared to Pop 2 and Pop 3. This reduction in genetic diversity levels is in accordance with the results of previous studies on durum wheat (Roussell *et al.*, 2004; Reif *et al.*, 2005) and might be explained by the introduction of high-

yielding dwarf varieties based on a limited number of key parents and that rapidly dominated the wheat germplasm base.

Table 2. Chromosome location, total number of alleles, gene diversity and PIC values of the 30 SSR markers used to study genetic diversity in the germplasm collection of durum wheat from Southern Italy.

Locus	Chr. Loc.	All. No.	Gene div.	PIC	Locus	Chr. Loc.	All. No.	Gene div.	PIC
TAGLUT	1AS	3	0.595	0.529	Xgwm164	1AS	3	0.459	0.362
Xgwm357	1AL	2	0.213	0.191	TAGLGAP	1BS	3	0.612	0.543
Xgwm268	1BL	5	0.726	0.680	Xgwm95	2AS	3	0.585	0.496
Xgwm448	2AS	6	0.756	0.720	Xgwm526	2BL	4	0.627	0.553
Xgwm374	2BS	3	0.090	0.087	Xgwm155	3AL	3	0.369	0.333
Xgwm369	3AS	5	0.619	0.544	Xgwm493	3BS	4	0.562	0.471
Xgwm389	3BS	4	0.460	0.424	Xgwm165-4A	4AS	2	0.498	0.374
Xgwm610	4AL	4	0.572	0.489	Xgwm6	4BL	7	0.813	0.788
Xgwm495	4BL	3	0.549	0.448	Xgwm165-4B	4BL	4	0.713	0.663
Xgwm415	5AS	2	0.254	0.222	Xgwm304	5AS	5	0.650	0.605
Xgwm234	5BS	3	0.204	0.191	Xgwm408	5BL	2	0.463	0.356
Xgwm169	6AL	2	0.292	0.249	Xgwm570	6AL	4	0.393	0.369
Xgwm518	6BS	5	0.752	0.714	Xgwm219	6BL	4	0.639	0.573
Xgwm282	7AL	6	0.632	0.562	Xgwm332	7AL	4	0.431	0.350
Xgwm46	7BS	4	0.582	0.493	Xgwm611	7BL	6	0.761	0.723
Mean		3.83	0.529	0.470					

Table 3. Summary statistics of diversity for 30 SSRs detected in 107 durum wheat accessions subdivided by plant height in Pop1, Pop 2 and Pop3.

Pop	n	N_a	N_e	I	H_e
1 (h≤70 cm)	20	2.667 a	1.932 a	0.713 a	0.439 a
2 (70cm<h<100cm)	63	3.767 b	2.298 b	0.908 bc	0.507 b
3 (h≥100 cm)	24	3.400 c	2.528 c	0.952 c	0.551 c
All	107	3.278	2.253	0.858	0.499

Wilcoxon signed-rank test, P < 0.001.

The loss of genetic diversity may indicate an erosion of alleles valuable for plant improvement and future demands of producers and consumers. Currently some of the limiting factors in the use of 'ex situ' collections are linked to the missing or incomplete characterization of collections. The level and the distribution of genetic diversity in the materials analysed in this study could be used as genetic criteria to protect these accessions in 'ex situ' collections, to further investigate their characteristics and to use them both for breeding systems and for the improvement of typical products.

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