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

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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 *N_a*, *N_e*, I et *H_e* 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.


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 prisms™ 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 MgCl2, 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 ($N_{ae}$), 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.

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

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.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Chr. Loc.</th>
<th>All. No.</th>
<th>Gene div.</th>
<th>PIC</th>
<th>Locus</th>
<th>Chr. Loc.</th>
<th>All. No.</th>
<th>Gene div.</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAGLUT</td>
<td>1AS</td>
<td>3</td>
<td>0.595</td>
<td>0.529</td>
<td>Xgwm164</td>
<td>1AS</td>
<td>3</td>
<td>0.459</td>
<td>0.362</td>
</tr>
<tr>
<td>Xgwm357</td>
<td>1AL</td>
<td>2</td>
<td>0.213</td>
<td>0.191</td>
<td>XAGLGAP</td>
<td>1BS</td>
<td>3</td>
<td>0.612</td>
<td>0.543</td>
</tr>
<tr>
<td>Xgwm268</td>
<td>1BL</td>
<td>5</td>
<td>0.726</td>
<td>0.680</td>
<td>Xgwm95</td>
<td>2AS</td>
<td>3</td>
<td>0.585</td>
<td>0.496</td>
</tr>
<tr>
<td>Xgwm448</td>
<td>2AS</td>
<td>6</td>
<td>0.756</td>
<td>0.720</td>
<td>Xgwm526</td>
<td>2BL</td>
<td>4</td>
<td>0.627</td>
<td>0.553</td>
</tr>
<tr>
<td>Xgwm374</td>
<td>2BS</td>
<td>3</td>
<td>0.090</td>
<td>0.087</td>
<td>Xgwm155</td>
<td>3AL</td>
<td>3</td>
<td>0.369</td>
<td>0.333</td>
</tr>
<tr>
<td>Xgwm369</td>
<td>3AS</td>
<td>5</td>
<td>0.619</td>
<td>0.544</td>
<td>Xgwm493</td>
<td>3BS</td>
<td>4</td>
<td>0.562</td>
<td>0.471</td>
</tr>
<tr>
<td>Xgwm389</td>
<td>3BS</td>
<td>4</td>
<td>0.460</td>
<td>0.424</td>
<td>Xgwm165-4A</td>
<td>4AS</td>
<td>2</td>
<td>0.498</td>
<td>0.374</td>
</tr>
<tr>
<td>Xgwm610</td>
<td>4AL</td>
<td>4</td>
<td>0.572</td>
<td>0.489</td>
<td>Xgwm6</td>
<td>4BL</td>
<td>7</td>
<td>0.813</td>
<td>0.788</td>
</tr>
<tr>
<td>Xgwm495</td>
<td>4BL</td>
<td>3</td>
<td>0.549</td>
<td>0.448</td>
<td>Xgwm165-4B</td>
<td>4BL</td>
<td>4</td>
<td>0.713</td>
<td>0.663</td>
</tr>
<tr>
<td>Xgwm415</td>
<td>5AS</td>
<td>2</td>
<td>0.254</td>
<td>0.222</td>
<td>Xgwm304</td>
<td>5AS</td>
<td>5</td>
<td>0.650</td>
<td>0.605</td>
</tr>
<tr>
<td>Xgwm234</td>
<td>5BS</td>
<td>3</td>
<td>0.204</td>
<td>0.191</td>
<td>Xgwm408</td>
<td>5BL</td>
<td>2</td>
<td>0.463</td>
<td>0.356</td>
</tr>
<tr>
<td>Xgwm169</td>
<td>6AL</td>
<td>2</td>
<td>0.292</td>
<td>0.249</td>
<td>Xgwm570</td>
<td>6AL</td>
<td>4</td>
<td>0.393</td>
<td>0.369</td>
</tr>
<tr>
<td>Xgwm518</td>
<td>6BS</td>
<td>5</td>
<td>0.752</td>
<td>0.714</td>
<td>Xgwm219</td>
<td>6BL</td>
<td>4</td>
<td>0.639</td>
<td>0.573</td>
</tr>
<tr>
<td>Xgwm282</td>
<td>7AL</td>
<td>6</td>
<td>0.632</td>
<td>0.562</td>
<td>Xgwm332</td>
<td>7AL</td>
<td>4</td>
<td>0.431</td>
<td>0.350</td>
</tr>
<tr>
<td>Xgwm465</td>
<td>7BS</td>
<td>4</td>
<td>0.582</td>
<td>0.493</td>
<td>Xgwm611</td>
<td>7BL</td>
<td>6</td>
<td>0.761</td>
<td>0.723</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>3.83</td>
<td>0.529</td>
<td>0.470</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Pop</th>
<th>n</th>
<th>Ns</th>
<th>Ne</th>
<th>I</th>
<th>Hs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (h≤70 cm)</td>
<td>20</td>
<td>2.667 a</td>
<td>1.932 a</td>
<td>0.713 a</td>
<td>0.439 a</td>
</tr>
<tr>
<td>2 (70cm&lt;h&lt;100cm)</td>
<td>63</td>
<td>3.767 b</td>
<td>2.298 b</td>
<td>0.908 bc</td>
<td>0.507 b</td>
</tr>
<tr>
<td>3 (h≥100 cm)</td>
<td>24</td>
<td>3.400 c</td>
<td>2.528 c</td>
<td>0.952 c</td>
<td>0.551 c</td>
</tr>
<tr>
<td>All</td>
<td>107</td>
<td>3.278</td>
<td>2.253</td>
<td>0.858</td>
<td>0.499</td>
</tr>
</tbody>
</table>

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.

References


