Improvement of oat hexaploid lines's groat nutritive value *via* hybridisation with tetraploid oat *A. magna*

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Abstract

For the aim of producing new oat lines with high nutritive value which will be conceived for human consumption, introgression of *A. magna* high groat protein content trait into common oat cultivars of *A. sativa*, through hybridisation, had been achieved. Derivative hybrids were subjected to pedigree selection and out of which, height lines have shown good agronomic performance and assessed for groat protein content. Protein analysis revealed that groat protein content of the derivative lines was improved by 4 to 56% compared to their hexaploid parents of common oats.

Keywords: Oats, tetraploid oat *A. magna*, Common oat *A. sativa*, hybridisation, groat protein content

{**Citation :** Saidi N., S. Saidi, A. Hilali, M. Benchekroun, C. Al Faiz, M. Bouksaim, N. Shaimi, A. Souihka, A. Salih Idrissi, F. Gaboune, G. Ladizinsky. Improvement of oat hexaploid lines's groat nutritive value *via* hybridisation with tetraploid oat *A. magna*. American Journal of Research Communication, 2013, 1(9): 126-135} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Recently, many epidemiological studies have revealed that regular consumption of oat whole grains reduces risks of various types of chronic diseases, such us cardiovascular disease, type 2 diabetes, some cancers (Donkor *et al.*, 2012). This is due to the high nutritive value of oats

which make this cereal a good source of vitamins, minerals and contains approximately twice the protein content of rice (Mohsin *et al.,* 2007). Oat caryopsis is rich in protein (16 – 18 %) and oil, in addition to vitamins of B complex, compared to other major cereals. Zhu and al. (2004) reported that both protein and oil groat contents are the most important oat quality traits. Because of its good nutritive value, oat breeding programmes are recently directed to grain production. Attempts have been made to use wild oat species to improve the grain quality of existing common oat cultivars. A tetraploid oat species *A. magna* has received considerable attention during last years, since it contains the tetraploid base which has served to the creation of the hexaploid cultivated forms (Leggett, 1992). In addition to be native of Morocco, more adapted to Moroccan soil and climate conditions and having good resistance to diseases, this taxa has a groat protein content which can reaching up to 30% (Welch *et al*., 2000; Loskutov, 2001) coupled with a great productive tillering (Loskutov, 2001). Moreover, *A. magna* has the particularity of being genetically close enough to the hexaploid oat species *A. sativa*, therefore any interspecific crosses with the hexaploid common oat might be successfull (Saidi and Ladizinsky, 2005). This hypothesis was previously confirmed by Ladizinsky and Fainstein (1977) that the improvement of the hexaploid oat protein groat content via introgression from tetraploids species *A. magna* is very promising to improve oat cultivation, because the genes which are controlling groat protein content are apparently located in homeologues chromosomes of the hexaploids and the tetraploid oats species. In order to improve the groat quality of some Moroccan hexaploid cultivars of *A. sativa*, the transfer of *A. magna*'s high groat protein to them was achieved through hybridisation work. The main objective of this study is to assess the groat protein content of the lines derivatives from different combinations of the interspecific crosses, since groat protein content is more often ranked as the most important trait among grain constituents due to its high nutritional value (Doehlert *et al*., 2001).

Material and Methods

Material

Two wild accessions of *A. magna* (158 and 169) collected in 1985 in different regions of Morocco, were involved in interspecific crosses with different Moroccan cultivars of *A. sativa* (Amlal, Ghali, Soualem, Tissir and Zahri). The cultivars were used as female parent in the first crossing cycle. The yielded hybrids were backcrossed to their hexaploid parents respectively (*A. sativa* x *A. magna*) x *A. sativa*). Ploidy analysis of the derivative hybrids was analysed and only hexaploid hybrids (2n=6x=42) were selected and had been subjected to pedigree selection until reaching genetic stability. In total, eight hexaploid lines were selected due to their good agronomic performance and analysed for groat quality.

Methods

Determination of the weight of one thousand Seeds (WTS)

For each line, 1000 seeds were counted in three replications and weighted individually. Average weight was determined and expressed in grams.

Determination of groat and hull proportions

Groat and husks proportions were carried out according to Doelehrt *et al*. (1999) and Hall *et al*. (2003). This method consists in counting and weighting 1000 seeds in three replicates and then proceed to their hand dehulling and to weight groat and hull separately. Groat and hull percentages were determined by measuring the mass of seed sample. Ratio of the groat or hull mass to the total sample mass times 100.

Determination of groat protein content

Determination of groat protein content consists in determining the total grain nitrogen using the classical Kjeldahl method (DIN ISO 15178, 2001). One gramme of oat flour (Pe) was weighted in two replicates and each sample was placed in a special container. One Kjeldahl tablet (0.3 g $CuSO_4$ + 3.4 g K_2SO_4) to catalyse the reaction, in addition to 12 ml of H_2SO_4 were added. The container was placed for 45 min in the mineralisation unit pre-heated at 420°C, until obtaining the green coloration. After cooling the mixture, 50 ml of distilled water was added. The containers in addition to the check (V_B) were placed in the distillation unit and 50 ml NaOH 40% was added. After distillating the residue for 5 min (200 ml of distillate) in 20 ml H_3BO_3 4% and added to Rm and Vb. Titration of the distillate was achieved using HCL 0.1 N (V_{HCL}).

Calculation methods for total proteins calculation

$$MAT = [(V_{HCL} - V_B) \times N_{HCL} \times 0.014 \times 6.25 \times 100] / (Pe \times MS)$$

$$MAT = [(V_{HCL} - V_B) \times 0.875] / (Pe \times MS)$$

Results and discussion

Weight of 1000 seeds (WTS)

The analysis of 1000 seeds for the derivative hybrids from all different combinations of (*A. sativa* x *A. magna*) x *A. sativa*, in comparison to their tetraploid and hexaploid parents, has shown variability of this character. For individual derivatives of [(Ghali x 158) x Ghali], WTS varies from 30 to 36 grams ranging between the WTS of the tetraploid parent *A. magna* 158 (WTS = 44 g) and that of the hexaploid parent *A. sativa* (Ghali) (WTS = 30g). Three genotypes had a WTS exceeding that of the hexaploid parent with WTS ranging from 31 to 36g (Fig. 1.1). Thus, the WTS and consequently the seed size of these later were improved through hybridization work.

For the combination [(Soualem 169) x Soualem], WTS of the unique derivative genotype was of 29 g, which is lower than that of the tetraploid parent *A. magna* 169 (WTS = 47g) as well as of the hexaploid parent *A. sativa* (Soualem) (WTS = 35g). Thus, no seed size improvement was achieved for the descendent genotype (Fig. 1.2.).

Analysis of WTS for the derivatives of [(Zahri x 169) x Zahri] has revealed that the WTS of the hybrid lines was lower than that of the hexaploid parent Zahri (WTS = 39g) and of the tetraploid parent *A. magna* 169 (WTS = 47g) (Fig. 1.3).

The only derived hybrid from the combination [(Tissir x 158) x Tissir] had a WTS of 36 g which is lower than that of tetraploid parent *A. magna* 158 (WTS = 44g) but exceeding the once of the hexaploid parent Tissir (WTS = 32 g). This indicated that seed size was improved for this genotype compared to its hexaploid parent (Fig 1.4).

According to the above results, we can deduct that seed size was improved for some derivative lines of the interspecific crosses between tetraploid and hexaploid species. This has resulted in the WTS increase especially for individuals derivative from the cross with *A. magna* 158 compared to once derivative from the cross with *A. magna* 169.

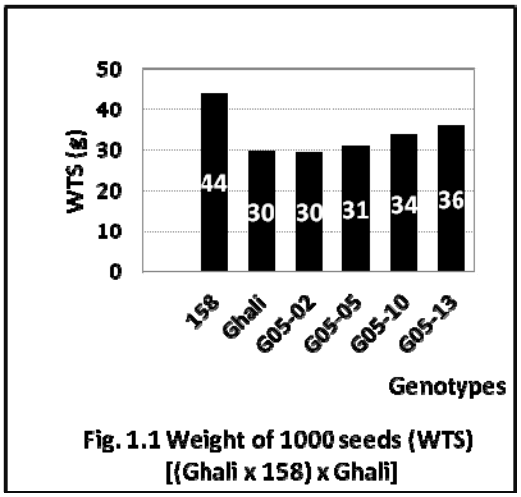

Fig. 1.1 Weight of 1000 seeds (WTS) [(Ghali x 158) x Ghali]

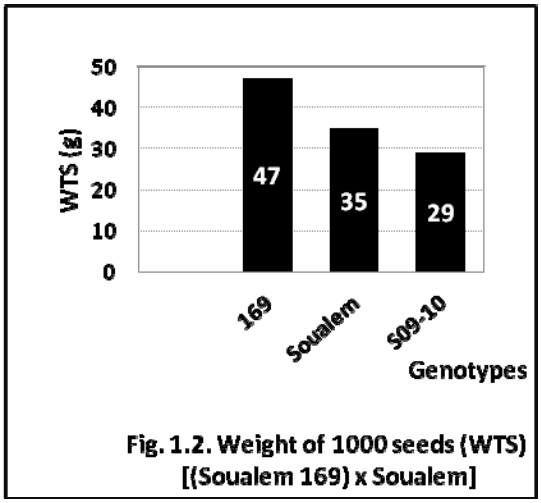

Fig. 1.2. Weight of 1000 seeds (WTS) [(Soualem 169) x Soualem]

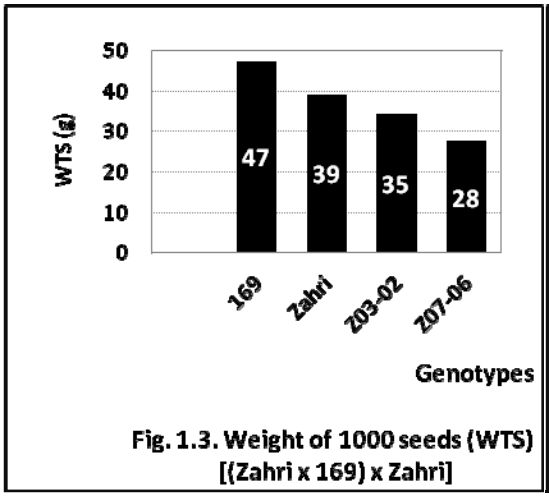

Fig. 1.3. Weight of 1000 seeds (WTS) [(Zahri x 169) x Zahri]

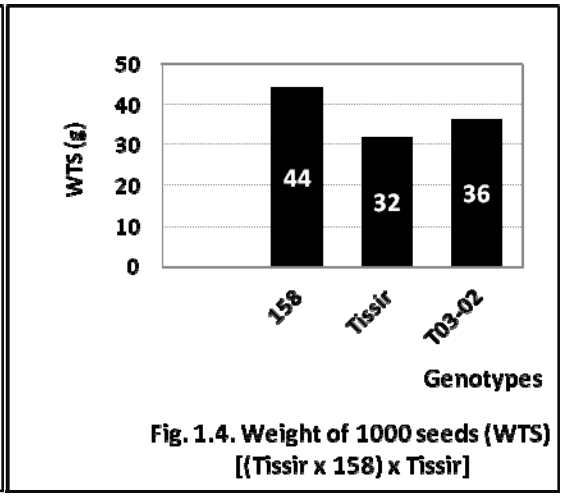

Fig. 1.4. Weight of 1000 seeds (WTS) [(Tissir x 158) x Tissir]

Fig. 1. Weight of one thousand seeds (WTS) (g) of the genotypes derivative from (*A. sativa* x *A. magna*) x *A. sativa*) combinations.

Groat and hulls proportions

For all coombinations, comparison of the multiple range test with the least significant difference method (LSD) has revealed a high significant difference (P < 0,01***) between the issued lines and both tetraploid and hexaploid parents for the studied traits.

Analysis of groat proportion compared to the total mass of seed sample for individuals derivative of [(Ghali x 158) x Ghali] has shouwn that gorat proportion varies from 61 to 72 %. Three individuals had a groat protportion greater and a hull proportion lower than that of the parental tetraploid *A. magna* 158 (54 % groat, 46 % hulls) and of their hexaploid parent Ghali (62 % groat, 38 % hulls). Thus, the highest groat proportion was obtained by the genotype G05-10 (72 % groat, 28 hulls), followed by G05-13 (71 % groat, 29 % hulls) then G05-05 (64% graot, 36 % hulls) (Fig. 2.1).

For both the two individuals maintained from the cross between *A. magna* and Zahri, groat proportion for genotype Z07-06 was of 74 % and therefore exceeding the once of the tetraploid parent *A. magna* 169 (56 % groat) and the hexaploid parent Zahri (71 % graot). Hulls proportion was determined for this genotype and was of 26 %, lower than the once of *A. magna* 169 (44 % hulls) and of Zahri (29 % hulls). For the other derivative genotype Z03-02, both groat and hulls prportions were not different from that of the hexaploid parent Zahri (Fig. 2.2).

For the genotype S09-10 derivative from cross between Soualem and *A. magna* 169, groat proportion was of 72 % and thus exceeding the ones of its both parents which are of 70 % and 56 % respectively. Hulls proportion for this individual was of 28 % lower than that of both parents which are of 30 % and 44% respectively (Fig. 2.3).

The maintained genotype from the cross involving Tissir and *A. magna* 158, T03-02 had a graot proportion of 74 % higher than the ones of *A. magna* 158 (54 %) and Tissir (69 %). Hulls proportion for this individual was of 26 % lower than the ones of both parents 46 % and 31 % respectively (Fig. 2.4).

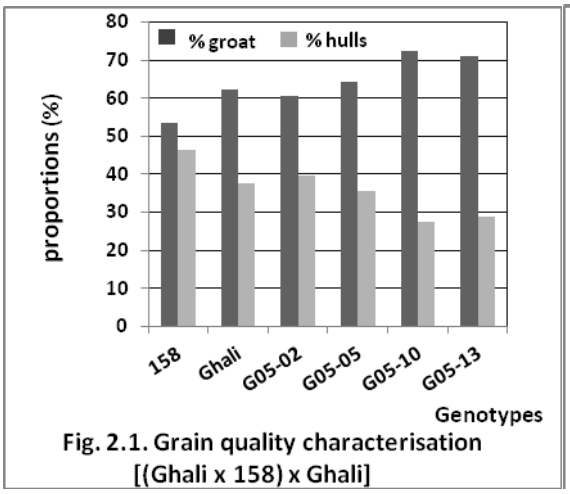

Fig. 2.1. Grain quality characterisation [(Ghali x 158) x Ghali]

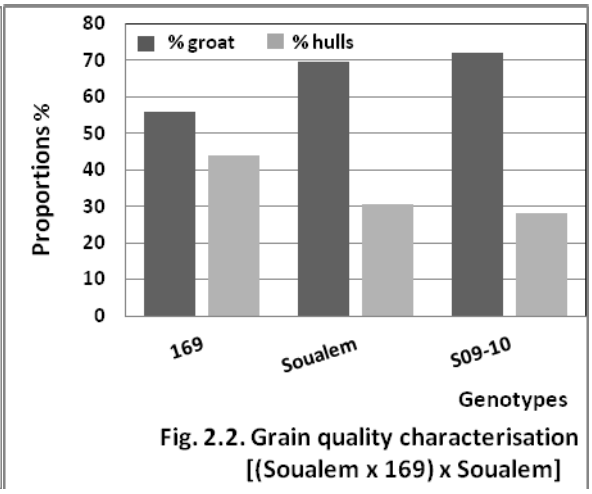

Fig. 2.2. Grain quality characterisation [(Soualem x 169) x Soualem]

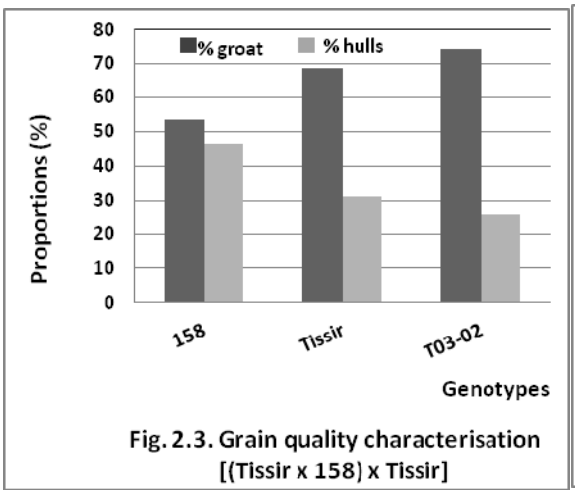

Fig. 2.3. Grain quality characterisation [(Tissir x 158) x Tissir]

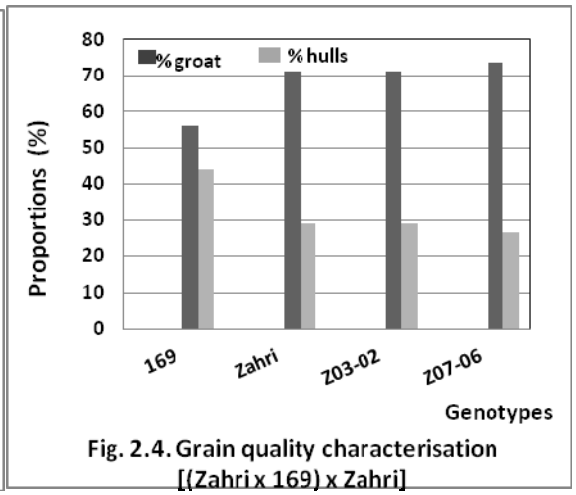

Fig. 2.4. Grain quality characterisation [(Zahri x 169) x Zahri]

Fig. 2. Proportions of groat, hulls and groat protein for derivative genotypes from different combinations of (*A. sativa* x *A. magna*) x *A. Sativa.*

Groat protein content

For all combinations, comparison of the multiple range test with the least significant difference method (LSD) has revealed a high significant difference (P < 0,01***) between derivative lines and their both tetraploid and hexaploid parents for this trait.

For cross [*A. sativa* (Ghali) x *A. magna* 158) x *A. sativa* (Ghali)], determination of groat protein content for derivatives lines has revealed that three individuals G05-10, GO5-13 and G05-02 had groat protein content of 16,71 %, 16,35% and 15,95% respectively, exceeding that of Ghali (15,32%) but lower than that of *A. magna* 158 (27,75%) (Fig. 3.1). Hence, trait was improved by 4 to 9 % for the issued lines.

For cross [*A. sativa* (Zahri) x *A. magna* 169) x *A. sativa* (Zahri)], the lines Z03-02 and Z07-06 derivatives of this cross involving Zahri and *A. magna* 169, groat protein content was of 17,08% and 14,25 %, respectively. Recorded protein content exceeds that of their hexaploid parent Zahri (10,93%) but lower when compared to than that of the tetraploid parent *A. magna* 169 (24,40 %) (Fig. 3.2). Thus, improvement of groat protein content in derivative lines reached 31 to 56 %.

Concerning [*A. sativa* (Soualem) x *A. magna* 169) x *A. sativa* (Soualem)] combination, the unique maintained derivative line S09-10 after selection, had a groat protein content of 16,19 %, exceeding that of Soualem (10,43%) but lower than that of *A. magna* 169 (24,4 %) (Fig. 3.3). Therefore, protein content in the maitained line was improved by 55 %.

For cross [*A. sativa* (Tissir) x *A. magna* 158) x *A. sativa* (Tissir)], after assessing the derivative lines throug pedigree selecion, only one line T03-02 was maintained and assessed for graot protein content which reached 18,37%. This content was found higher than that of its hexaploid parent Tissir (14,03%) and lower than that of its tetraploid parent *A. magna* 158

(24,75 %) (Fig. 3.4). Therefore, groat protein content was improved by 31 % for this issued line.

However, lines derivatives from the crosses realised between Tissir or Ghali cultivars with *A. magna* 158, respectively, groat protein content has been increased by 4 to 31 %. In the other hand, lines derivatives from the crosses involving Soualem or Zahri with *A. magna* 169 respectively, groat protein content was increased by 55% to 56 % despite that the used hexaploid parents for these combinations had the lowest groat protein content (an average of 12 %) compared to the other hexaploid parents Tissir and Ghali (an average of 17 %) used in the first crosses with *A. magna* 158.

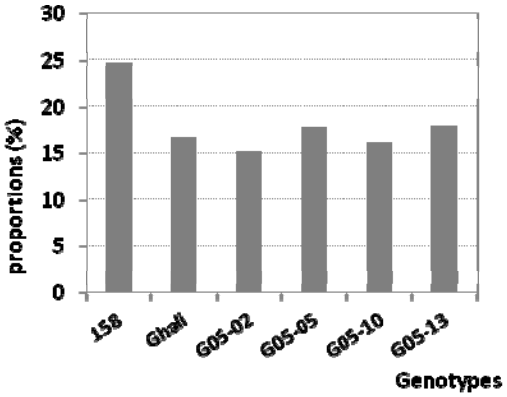

Fig. 3.1. Groat protein content in derivatives of [(Ghali x 158) x Ghali]

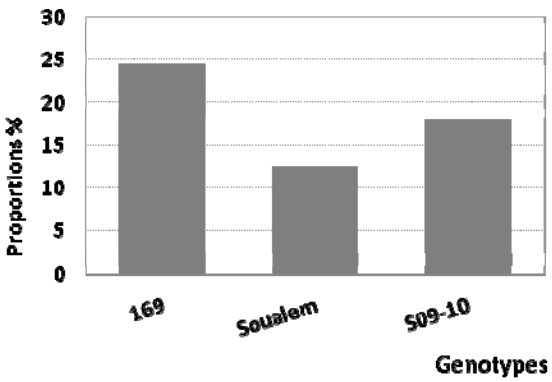

Fig. 3.2. Groat protein content in derivatives of [(Soualem x 169) x Soualem]

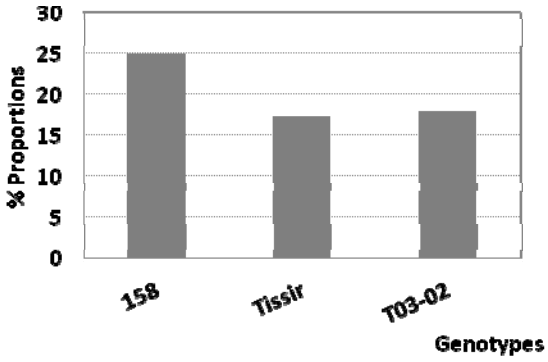

Fig. 3.3. Groat protein content in derivatives of [(Tissir x 158) x Tissir]

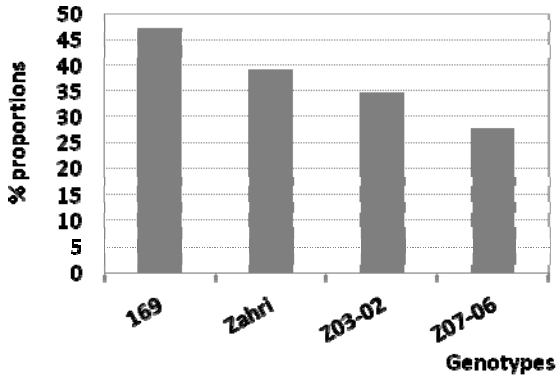

Fig. 3.4. Groat protein content in derivatives of [(Zahri x 169) x Zahri]

Fig. 3. Proportions of groat protein content (%) in the derivative genotypes issued from different combinations of (*A. sativa* x *A. magna*) x *A. Sativa.*

The most important grain quality traits for oats are weight test, groat proportion and groat constitution mainly oil and protein contents (Doehlert *et al.*, 2001).

Analysis of WTS for the derivative hybrids was increased compared to that of hexaploid parents. According to Doehlert *et al.*, (2001), groat proportion is a determinant trait for oat quality economic value and it is positively correlated with seed yield. Analysis of this trait for

the progeny, compared to both hexaploid and tetraploid parents, has shown an increase of groat proportion and a decrease of hull proportion. This leads to conclude that high groat proportion of tetraploid parents was transferred to all derivative hybrids of (*A. sativa* x *A. magna*) x *A. sativa*. This confirms what was reported by Doehlert *et al*., (1999) that groat proportion is an inheritable quantitative trait having a large heritability varying from 36 to 92%. It was reported by Welch *et al.* (2000) that oat grain quality is also determined by groat protein and oil contents. Thus, our analysis was limited to groat protein content since it is more often ranked as the most important trait among grain constituents due to its high nutritional value (Doehlert *et al*., 2001). This leads to conclude that in general, targeted trait was successfully transferred from the tetraploid parent *A. magna* to the progeny through hybridisation work. This confirms what was reported by Ladizinsky and Fainstein (1977), that the improvement of groat protein content of cultivated oats by transferring this trait from tetraploid species *A. magna* to hexaploid cultivars, is very promising because the genes controlling this trait are apparently localised on homologous or homeologus chromosomes of both hexaploid and tetraploid species. According to Marshall *et al.* (1992) and Zhu *et al.* (2004), these genes have a large additive action with a partial to total dominance for low groat protein content. Hence, the genomic regions associated to high groat protein content and their specific molecular markers are of great use for genetic control of this trait, and will provide assistance to breeders in order to manipulate this trait aiming the improvement of oat cultivation (Zhu *et al.*, 2004).

Since the main objective of this study is to develop new hexaploid lines having a high grain quality especially high groat protein content exceeding that of cultivated hexaploids, according to the above results, seven lines derivatives of (*A. sativa* x *A. magna*) x *A. sativa* were maintained (G05-10, G05-13, G05-02, S09-10, T03-02, Z03-02 and Z07-06) and will be suggested for human consumption.

Conclusion

Improving oat cultivation by enlarging the genetic basis of existing cultivated oat's cultivars is possible by transferring through hybridisation, valuable traits from wild relative species of oat, which are genetically close enough to common oat such as the wild tetraploid oat species *A. magna*. Compared to their hexaploid parents, groat protein content for the lines issued from the crosses between tetraploid accessions of *A. magna* and Moroccan hexaploid cultivars had been inhanced by 4 to 56 %. Our results showed that higher groat protein content trait was successfully transferred from tetraploid parent *A. magna* to the progeny through hybridisation work. Therefore, seven yielded hexaploid lines having good groat protein content were selected and can be conceived for human consumption. However, these lines still need to be subjected to more advanced food technology analysis in order to assess their real nutritive value.

Acknowledgments

This work was undertaken within the framework of the project financed by USAID, Project No. TAMOU 97-053. Great thanks go to Dr. Jean Koening from INRA-Clermont-Ferrond, France, for helping undertaking the chemical analysis. My special thanks go to Mrs. N. Shaimi, Mr. A. Souihka, Mr. A. Salih Idrissi and Mr. A. Abtah, INRA-CRRA, Rabat, Morocco, for their help over years of selection work, without their support this work could not be realised. My special thanks go to Mrs. R. Kallida, INRA, Prof. I. Loskutov, Vavilov Institute of Plant Industry, Russia, and Prof. M. Dost, FAO expert consultant RNE, Egypt, for their kind help in reviewing the manuscript.

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