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ASSESSMENT OF GROAT NUTRITIONAL ASPECT OF NEW DEVELOPED OAT HEXAPLOID LINES THROUGH INTERSPECIFIC CROSS WITH THE TETRAPLOID OAT *A. MURPHYI*

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ABSTRACT

Attempts have been made, to develop high groat protein content lines of hexaploid oat through hybridization work, aiming the transfer the tetraploid oat species A. murphyi's high groat protein content into three Moroccan common oat cultivars. Derivative hybrid lines were subjected to pedigree selection, which yielded ten lines showing a good agronomic performance. Since the derivative lines will be conceived for human consumption, selected lines were assessed for groat protein content. Protein analysis revealed that this trait was improved for the developed lines from 1 to 18% in comparison to their hexaploid parents, respectively.

KEYWORDS: Oats, Tetraploid Oat *A. Murphyi*, Common Oat *A. Sativa*, Hybridisation, Groat Protein Content

INTRODUCTION

Known to be the most popular cool-season cereal species grown for forage [4], traditional breeding programmes were emphasising on the determination of agronomic performance of oats mainly high grain yield, test weight, straw strength and tolerance/ resistance to diseases which threaten oat cultivation. However, during the last decades, more attention was devoted to other characters, mainly grain quality such as high groat protein content [12]. This is because many epidemiological studies have revealed that regular consumption of oat, whole grains reduces risks of various types of chronic diseases, such as cardiovascular disease, type 2 diabetes and some cancers [9]. Oats have considerable amounts of high-quality protein [26], which is approximately twice the protein content of rice, and are a good source of vitamins and minerals [18]. Furthermore, oats have a good taste, dietetic properties and an activity stimulating metabolic changes in the body [2] and containing unique antioxidants (avenanthramides), various phenolic compounds [20], in addition to be beneficial as a gluten-free diet suggested for individual genetically susceptible for gluten [19 ; 1]. For all these features, demands for oats for human consumption have increased during the last decades. Therefore, most of the breeding programmes are currently directed towards grain yield improvement rather than forage yield in order to release new cultivars with more protein of better quality in order to satisfy these demands [24]. A wild tetraploid oat species *Avena murphyi* Ladiz. (2n=4x=28) endemic to South of Spain, but encountered also in the North of Morocco [13 ; 15] was found to be interesting since its groat protein content can reach up to nearly 40% and therefore higher than that of the existing common oat cultivars [25 ; 16]. As it was reported by [3] that the value of introgressing wild oat germplasm into the

cultivated oat gene pool, in order to improve traits of economic importance, has been documented. Attempts have been made by the National Institute for Agricultural Research (INRA-Morocco) to exploit this valuable trait of this taxon to increase the nutritive value, mainly groat protein content, of three local common oat cultivars through hybridization work, aiming the development of new rich protein groat cultivars which can be conceived for human consumption. As it was reported by [23], groat protein content is ranked as the most important trait among grain constituents due to its high nutritional value. Therefore, this study aims to assess the groat protein content of the new developments hexaploid lines, derivatives from different combinations of the interspecific crosses.

MATERIAL AND METHODS

Material

Two wild accessions of *A. murphyi* Ladiz. (45-55 and 50-52) collected in 1985 in the South of Spain and Northern region of Morocco, were involved in interspecific crosses with three Moroccan cultivars of *A. sativa* (Amlal, Soualem and Tissir). The cultivars were used as female parent in the first crossing cycle. F1 hybrids were backcrossed to their hexaploid parents, respectively. The BC1 hybrids have been subjected to pedigree selection until reaching genetic stability [22]. Ten hexaploid lines were selected according to their agronomic performance and analyzed 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 [11].

Determination of Groat and Hull Proportions

Determination of the groat and husks proportions were released, according to [7] and [10]. This method consists in counting and weighting one thousand seeds in three replicates and then proceeds to their hand dehulling and to weight groat and hull separately. Groat and hull percentages were determined by measuring the mass of seed sample. The 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 Kjeldhal method [6]. One gramme of oat flour (Pe) was weighted in two replicates and each sample was placed in a special container. One Kjeldhal tablet (0.3 g CuSO4 + 3.4 g K2SO4) in addition of 12 ml of H2SO4 was added. The container was placed for 45 min in the mineralization 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 (VB) were placed in the distillation unit and 50 ml NaOH 40% were added. After distillation the residue for 5 min (200 ml of distillate) in 20 ml H3BO3 4% and added to Rm and Vb. Titration of the distillate was carried out using HCL 0.1 N (VHCL).

Calculation Method for Total Proteins

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

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

RESULTS AND DISCUSSIONS

Weight of 1000 Seeds (WTS)

Analysis of 1000 seeds for the derivative hybrids from different combinations of (*A. sativa* x *A. murphyi*) x *A. sativa* has revealed that individuals issued from combination A7 (Amlal x P45-55) x Amlal had a WTS ranging from 36 g to 39 g. Hence, this weight is lower than that of both tetraploid parent P45-55 (52 g) and hexaploid parent Amlal (42 g) (Figure 1).

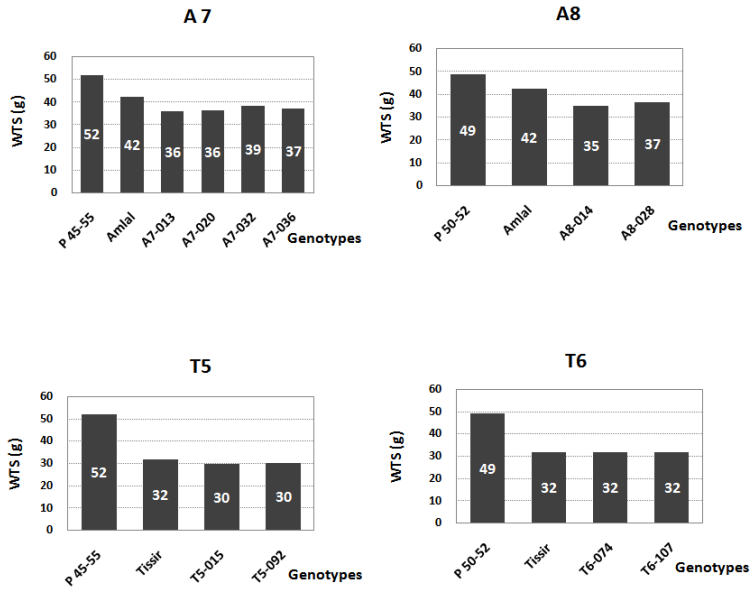

Figure 1: Weight of One Thousand Seeds (WTS) of the Genotypes Derivative from (*A. sativa* x *A. murphyi*) x *A. sativa*) Combinations

T5 (*A. sativa* (Tissir) x *A. murphyi* 45-55) x *A. sativa* (Tissir)

T6 (*A. sativa* (Tissir) x *A. murphyi* 50-55) x *A. sativa* (Tissir)

A7 (*A. sativa* (Amlal) x *A. murphyi 45-55*) x *A. sativa* (Amlal)

A8 (*A. sativa* (Amlal) x *A. murphyi 50-52*) x *A. sativa* (Amlal)

For combination A8 (Amlal x P50-52) x Amlal, WTS of the two maintained individuals varied from 35 to 37 g, lower than that of the tetraploid parent P50-52 (49 g) and the hexaploid parent Amlal (42 g) (Figure 1).

Derivative hybrids from the cross T5 (Tissir x P45-55) x Tissir had a WTS of 30 g lower than that of both Tissir (32 g) and of P45-55 (52 g) (Figure 1). As for cross T6 (Tissir x P50-52) x Tissir, derivative hybrids had a WTS of 32 g similar to that of Tissir but lower than that of P 50-52 (Figure 1). In general, crosses between hexaploid cultivars Amlal / Tissir and the tetraploid accessions of *A. murphyi* P45-55 and P50-52 respectively, had yielded individuals with a WTS lower than that of the tetraploid parents and lower or equal to that of their hexaploid parents.

Groat and Hulls Proportions

For all combinations A7, A8, T5 and T6, comparison of the multiple range test with the least significant difference method (LSD) has revealed a high significant difference ($P<0.001$) between derivative individuals and their parents respectively concerning these traits.

Analysis of the groat and hull proportions for derivative lines of the combination A7, realized between Amlal and P45-55, revealed that groat proportion has reached 68 to 74 %, exceeding that of both P45-55 (58 %) and Amlal (66 %). Also, hull proportions varied from 26 % to 32 % lower than that of both P45-55 (42 %) and Amlal (34 %) (Figure 2).

For combination A8, performed between Amlal and P50-52, the two derivative individuals had a groat proportion of 67 to 70 %, respectively, compared to that of Amlal (66 %). The derivative line A08-14 has a groat proportion of 70 % compared to that of the tetraploid parent P50-52 (69 %). Hulls proportion of the two derivative lines ranged from 30 to 33% and hence lower than that of the hexaploid parent Amlal (34 %) (Figure 2).

The combination T5, undertaken between Tissir and P45-55, the derivative lines T05-15 and T05-92 revealed a groat proportion of 66 % to 70 %, respectively, exceeding that of their tetraploid parent P45-55 (58 %). Only the line T05-92 had a groat proportion exceeding that of the hexaploid parent Tissir (69 %). As for the hulls proportion of both issued lines, it ranged from 30 to 34 % and hence lower than that of P45-55 (42%). For line T05-15, this trait was off 34 %, exceeding that of Tissir (31 %). For line T05-92, hulls proportion was off 30 % and therefore lower than that of the hexaploid parent Tissir (Figure 2).

The combination T6, which involved Tissir and P50-52, had yielded two lines T06-74 and T06-107 presenting a groat proportion of 62 % and 71 %, respectively. The T06-74 had a graot proportion (71 %) exceeding that of both parents, while that of T06-107 (62 %)was lower compared to its both parents (Figure 2).

Hulls proportion for T06-74 (29 %) was lower than that of its both parents (31 %) For T06-107 recorded hulls proportion was higher (38 %) than that of both parents. (Figure 2).

In general, for the crosses realized between Amlal or Tissir and both tetraploid accessions of *A. murphyi* P45-55 and P50-52, respectively, there was an improvement of groat proportion and a decrease of hulls proportion mainly for the derivatives of the crosses with P45-55.

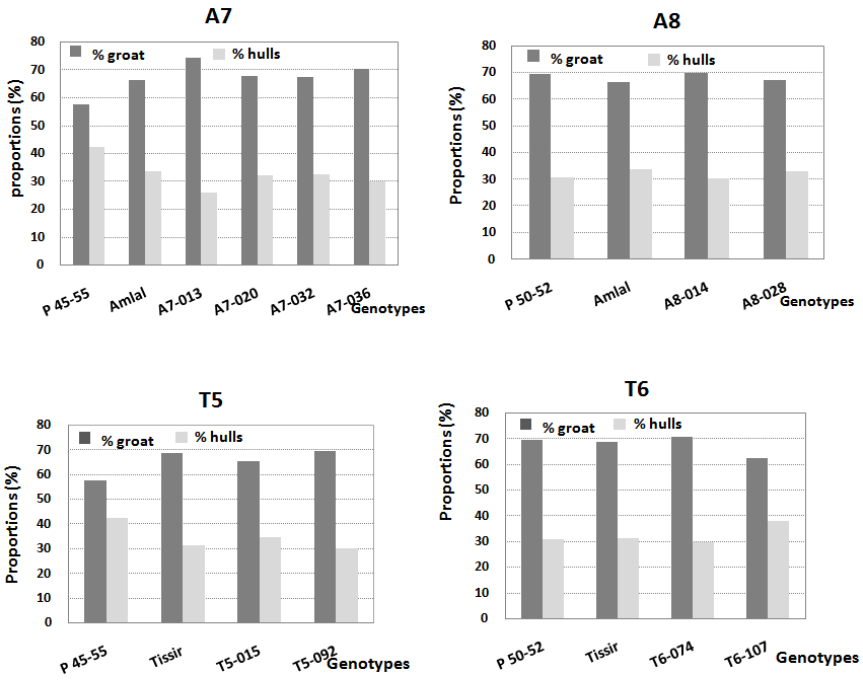

Figure 2: Proportions of Groat and Hulls and for Derivative Lines from Different Combinations of (*A. sativa* x *A. murphyi*) x *A. sativa*

T5 (*A. sativa* (Tissir) x *A. murphyi* 45-55) x *A. sativa* (Tissir)

T6 (*A. sativa* (Tissir) x *A. murphyi* 50-55) x *A. sativa* (Tissir)

A7 (*A. sativa* (Amlal) x *A. murphyi 45-55*) x *A. sativa* (Amlal)

A8 (*A. sativa* (Amlal) x *A. murphyi 50-52*) x *A. sativa* (Amlal)

Groat Protein Content

For all combinations T5, T6, A7 and A8, comparison of the multiple range test with the least significant difference method (LSD) has revealed the existence of a high significant difference ($P<0.001$) between the derivative hybrids and their parents respectively regarding this trait.

Analysis of groat protein content for derivative individuals of the combination A7 realized between Amlal and P45-55 has shown that groat protein content of three hybrids A07-13 (14,61 %), A07-20 (14,54 %) and A07-36 (14,52 %) was improved by 3 to 4 %, compared to the once of their hexaploid parent Amlal (14,03 %) (Figure 3).

For combination A8 involving Amlal and P50-52, groat protein content for the issued hybrids A08-14 (13,79 %) and A08-28 (12,98 %), was lower than that of their both parents P50-52 (18,39 %) and Amlal (14,03 %) and therefore, no genetic enhancement regarding this trait was achieved through this cross (Figure 3).

For T5 combination involving Tissir and P45-55, the groat protein content of the hybrids was lower than that of their tetraploid parents P45-55 (23.69 %). In comparison to their hexaploid parent Tissir (14 %), the two derivative hybrids T05-92 and T05-15 had a groat protein content of 16,59 % and 15,94 %, respectively, enhanced in comparison to Tissir from 14 to 18 % (Figure 3).

For T6 combination undertaken between Tissir and P50-52, the groat protein content of the derivative hybrids T06-74 (14,18 %) and T06-107 (9,97 %) was lower than that of tetraploid parent P52-55 (18 %). Only one hybrid T06-74 had a groat protein content of 14,18 % enhanced by 1 % than that of Tissir (Figure 3).

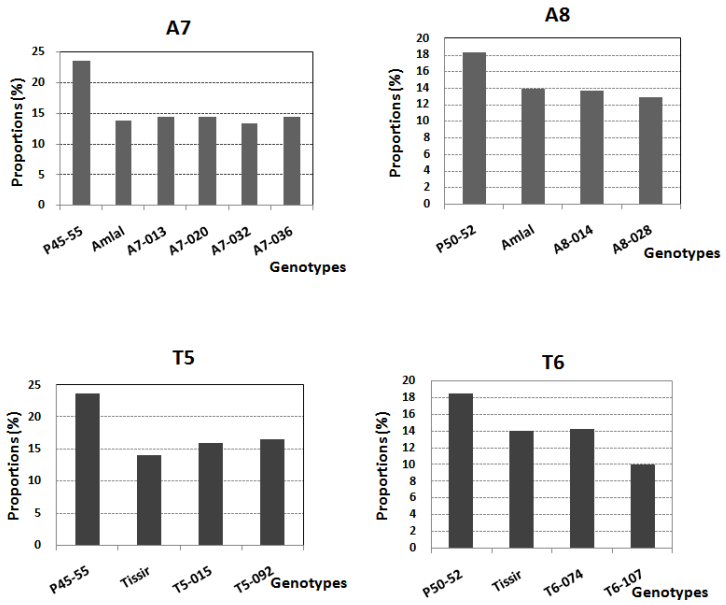

Figure 3: Proportions of Groat Protein Content (%) in the Derivative Lines Issued from Different Combinations of (*A. sativa* x *A. murphyi*) x *A. sativa*

T5 (*A. sativa* (Tissir) x *A. murphyi* 45-55) x *A. sativa* (Tissir)

T6 (*A. sativa* (Tissir) x *A. murphyi* 50-55) x *A. sativa* (Tissir)

A7 (*A. sativa* (Amlal) x *A. murphyi 45-55*) x *A. sativa* (Amlal)

A8 (*A. sativa* (Amlal) x *A. murphyi 50-52*) x *A. sativa* (Amlal)

According to the above results, we can conclude that no groat protein content improvement was noticed for the derivative hybrids of the cross A8 realised between hexaploid cultivar Amlal and the tetraploid parent P50-52. However, for the cross A7 achieved between Amlal and P45-55, for most of the yielded hybrids there was an enhancement of the groat protein content of 3 to 4 %. On the other hand, for one derivative individual from the cross T6 realized between Tissir and the tetraploid accession P50-52, there was an increase of around 1 % of groat protein content compared to its hexaploid parent. However, the important genetic enhancement of the groat protein content was achieved through the cross T5, which involved Tissir and P45-55. The groat protein content of the derivative lines was enhanced by 14 to 18 % compared to its hexaploid parent. Thus, for the crosses realized between the tetraploid parent P45-55 with either hexaploid cultivars Amlal or Tissir, we have succeeded in enhancing the groat protein content of the progeny.

Reminding what was quoted by [8], weight test, groat proportion and its constitution mainly oil and protein contents are the most important grain quality traits for oats. Thus, derivative hybrids yielded by different cross combinations between hexaploid cultivars and wild tetraploid accessions of *A. murphyi* respectively, were analyzed for the targeted traits.

Derivative lines issued from different combinations of (*A. sativa* x *A. murphyi*) x *A. sativa* cross had shown a WTS lower or equal to that of the hexaploid parents. Thus, no genetic improvement for this trait was achieved for the progeny, compared to the lines, derivatives of the crosses realized with *A. Magna*, for which this trait was improved [23]. This leads to conclude that *A. Magna* is genetically close enough to the cultivated common oat *A. sativa* rather than any other oat species [21].

Groat proportion is a determinant trait for oat quality economic value and it is positively correlated with seed yield [8]. Thus, the derivative individuals of (*A. sativa* x *A. murphyi*) x *A. sativa*, groat proportion was improved for only the hybrid derivative of the combinations that involved Amlal and the tetraploid accessions of *A. murphyi*. However, in comparison to the derivative individuals from the combinations of the cross (*A. sativa* x *A. Magna*) x *A. sativa* previously achieved under the same environmental conditions by [23], there was an increase of groat proportion and a decrease of hull proportions in comparison to their both hexaploid and tetraploid parents. This leads to conclude that the high groat proportion of the tetraploid parents were transferred to all derivative hybrids of (*A. sativa* x *A. Magna*) x *A. sativa*, and to half of the progeny of the cross (*A. sativa* x *A. murphyi*) x *A. sativa*. This confirms what was reported by [7], that groat proportion is an inheritable quantitative trait having a large heritability varying from 36 to 92%.

The nutritionally desired constituents of oats are concentrated in the groat [5]. In addition to groat proportion, oat grain quality is also determined by groat protein and oil contents [25]. Groat protein content is more often ranked as the most important trait among grain constituents because of its high nutritional value [8]. Thus, analysis of groat protein content of the derivative individuals of the cross (*A. sativa* x *A. murphyi*) x *A. sativa*, we have succeeded in increasing the groat protein content of the derivative hybrids by 1 % to 18 %. This leads to conclude that the targeted trait was successfully transferred from the tetraploid parent *A. murphyi* to the progeny through hybridization work. This confirms

what was reported by [14], that the improvement of the groat protein content of cultivated oats by transferring this trait from either the tetraploid species *A. murphyi* to the hexaploid cultivars, is very promising because the genes controlling this trait are apparently localized on homologous or homologous chromosomes of both hexaploid and tetraploid species. According to [17] and [27], these genes have a large additive action with a partial to total dominance for low groat protein content. Hence, the genomic regions associated with 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 [27].

Reminding that the main objective of this study is to develop new hexaploid lines, having high grain quality, especially high groat protein content exceeding that of the cultivated hexaploid cultivars; and according to the above results, 6 lines derivative of the cross (*A. sativa* x *A. murphyi*) x *A. sativa* namely A07-13, A07-20, A07-36, T05-15, T05-92 and T06-74 were maintained. Because of their groat protein content which exceeded that of their hexaploid parents, the maintained lines can be suggested for being conceived for human consumption.

CONCLUSIONS

Wild relative species are a mine of valuable genes, which can be exploited by hybridization, aiming the improvement of a targeted cultivation. The obtained results from the undertaken hybridization work involving accessions of the wild tetraploid oat of *A. murphyi* and the different hexaploid oat cultivars of *A. sativa* respectively confirmed this hypothesis. The targeted trait by this study was the high groat protein content of the tetraploid parent. Assessment of the derivative hybrids yielded by different combinations for groat protein content has revealed an acceptable degree of improvement of this trait ranging from 1 to 18% compared to their hexaploid parents. This result is encouraging to boost new breeding programmes, targeting the development of new oat cultivars with high groat nutritive value and therefore responding to the accurate demand of the populations to use oats for human consumption. Therefore, the genetic progress achieved by *A. murphyi* remains relatively lower than that achieved by *A. Magna* as reported by [23]. Furthermore, the previous hypothesis stating that *A. Magna* is close enough to the cultivated oat of *A. sativa* rather than *A. murphyi* is again confirmed by this study.

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