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# Improving Groat B-Glucan Content of Developed Hexaploid Oat Lines Derivative of Interspecific Crosses

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**Abstract**
Oat grains, untapped reservoir of nutrients and phytochemicals, are very beneficial for food and feed. β-glucan content of sixteen derivative hexaploid lines, derivative of interspecific crosses between two tetraploid oat species *A. magna* and/or *A. murphyi* with five Moroccan hexaploid oat cultivars of *A. sativa,* aiming the improvement of their groat nutritive value, was accurately assessed. The new developed lines as well as their hexaploid oat parents were tested for agronomic performance under Moroccan climatic conditions in two different locations. Groat's composition analysis has revealed that β-glucan content ranged from 1,37% to 6,05%. Significant β-glucan contents were detected for the cultivar Zahri (6,05±1,19) followed by the lines *A. magna* $F_{11-4}$ (magxsat) (5,77±0,20)%, $F_{11-5}$ (magxsat) (5,79±0,31)%, and $F_{11-8}$ (magxsat) (5,55±0,41)%. The obtained results indicated that these lines are very promising. Furthermore, substantial differences among cultivars and lines over locations were observed and therefore, the genetic variability, the environmental conditions and their interaction has exerted statistically high significant effects on the groat β-glucan content ($p < 0,0001$). Thus, four among sixteen assessed lines which present high β-glucan content could be conceived for human consumption. These results confirm the success of acheived crosses and enhances the valorization of oats owing to its high content in β-glucans and other nutraceutical substances.
**Keywords:** Oat, β-glucan content, tetraploid oat, *A. magna*, *A. murphyi*, hexaploid oat, *A. sativa*, genotype x environmental conditions

## 1. Introduction

Some cereal grains and fungi, containing dietary fibres, hold an important economic commodity worldwide due to their particular chemical health promoting components (Butt *et al.*, 2008), among these cereal grains, oat (*Avena sativa* L.) ranking as sixth in cereal world production (Gujral *et al*., 2011). It is well known over the last decades that oat grain and its derived products proofed their qualification and offer multiple human health claims (Mckevith, 2004 ; Nakurte *et al*., 2013 ; Chang *et al.*, 2013).

Oat is considered as an exceptional flavorful cereal with high nutritional value arising from a high content of functional proteins, high portion of unsaturated fatty acids, an important source of B complex vitamins, etc. (Butt *et al.*, 2008). In addition, oat is characterized by the combination of manifold bioactive compounds such as antioxidants, polyphenols and heart healthy soluble dietary fibre such as β-glucan, which makes it a profitable dense matrix for human health (Mellen *et al.,* 2008 ; Gambus *et al.,* 2011). There is no doubt that oat, the richest, inexpensive and natural source of essential nutrients, can contribute, by a large amount of the accessible total protein and fiber intake, by general public as nutritional supplements and food ingredients (Hozova and Sturdik., 2005 ; Klose and Arendt., 2012).

In many breeding programs, untapped germplasm with different ploidy levels or wild progenitors are used as donors for specific alleles to develop new hexaploid oat lines, by recovering valuable hidden alleles from wild species or gene bank accessions through interspecific crosses (Ladizinsky, 2012). Regarding oat breeding, wild oat germplasm potential arising from its wide adaptability to different soil and climatic conditions, in addition to the number of traits determining high productivity and quality including resistance to pathogenic agents (Kole, 2011). Hence, the use of wild oat species is a promising way to improve the performance of Moroccan cultivars. Two species of wild oats tetraploid *A.murphyi* and *A. magna* are particularly advantageous, utilized to introduce new genetic diversity into the oat gene pool, necessary for the selection of the most interesting varieties for Morocco. Owing to their exceptionally high protein seeds, oil and to their endemism, both species are well adapted to the Moroccan climate and are genetically similar to *A. sativa* (Loskutov 2001). This study aimed to assess the groat β-glucan content of the derivative hexaploid oat lines of interspecific

 

crosses realized between Moroccan hexaploid cultivars of A. sativa and the wild tetraploid oat species *A.magna* and *A.Murphyi* for the aim of broadening their variability of groat β-glucan content.

## 2. Materials and methods

### 2.1 Plant material

For the experimental design, the used plant material concerned six Moroccan hexaploid oat cultivars (five of *A. sativa* L. and one cultivar of A.nuda, all registered in the Moroccan Official Catalogue), in addition to 2 accessions of tetraploid oat A. magna, two accessions of tetraploid oat A. murphyi, and sixteen cutivars of improved hexaploid oat of A. sativa, derivatives from interspecific crosses previously achieved between Moroccan hexaploid oat cultivars of *A. sativa* and wild accessions of tetraploid oat *A magna* Murphy and Terrell and A. *murphyi* Ladiz, respectively. (table 1). This breeding programme is conducted by the the National Institute for Agricultural Research (INRA), Rabat (Saidi, 2015). The experiments were conducted in trials following two randomized complete blocks in two known locations (Marchouch and Allal Tazi) (Saidi, 2015).

Table 1. list of crossbreeding achieved

| Hexaploid Parents | Tetraploid Parents | | Progeny |
|---|---|---|---|
| | *A. magna* | *A. murphyi* | |
| Amlal | | $P_{35-42}$ | $F_{10-1}$;$F_{10-2}$; $F_{10-3}$; $F_{10-4}$ |
| | | $P_{50-52}$ | $F_{10-5}$; $F_{10-6}$ |
| Tissir | | $P_{35-42}$ | $F_{10-7}$; $F_{10-9}$ |
| | | $P_{50-52}$ | $F_{10-8}$ |
| | $P_{1-1}$ | | $F_{11-5}$ |
| Ghali | $P_{1-1}$ | | $F_{11-1}$; $F_{11-2}$; $F_{11-3}$; $F_{11-8}$ |
| Soualem | $P_{1-6}$ | | $F_{11-4}$ |
| Zahri | $P_{1-6}$ | | $F_{11-7}$ |

Location 1: The Marchouch experimental station is located 68 km SE Rabat, Morocco (Longitude: 6° 71' W and latitude: 33° 60' N). Its altitude is of 410 m, an average annual rainfall of 407 mm. Marchouch is characterised by a rich black and crumbling fertile soil very suitable for cropping cereals and legumes.

Location 2: The experimental station of Allal Tazi is located at 94 km NE Rabat, Morocco (Longitude: 6° 19' W and latitude: 34° 31' N). Its altitude is of 10,5 m and an average annual rainfall of 660 mm.

### 2.2 Reagents and standards

Sodium dihydrogen orthophosphate dihydrate ($NaH_2PO_4$. $2H_2O$), sodium azide ($NaN_3$), glacial acetic acid, sodium hydroxide and ethanol were of analytical grade and obtained from Sigma-Aldrich. The Mixed-Linkage β-glucan Assay Kit used in the current research was procured from Megazyme (Megazyme International Ltd., Wicklow, Ireland).

### 2.3 Samples preparation

Assessed material was a harvest of 2012/2013 growing season and were processed for pre-cleaning, drying and storage. Whole grains were milled after drying in an oven at 40°C for 24 hr, using an ultra-centrifungal mill (UDY Cyclone) equipped with a 0.5 mm sieve. This mill allows nearly complete sample recovery.

### 2.4 Dry matter determination

This parameter was determined by oven drying samples at 80 °C for 24 hrs. Presented results are reported according to a dry weight basis (Brunner and Freed, 1994).

### 2.5 Assessment of β-glucan content

The content of soluble β-glucan was evaluated enzymatically according to AACC (Method 32-23), AOAC (Method 995-16) and ICC (Method 168), using a mixed β-glucan linkage kit (Megazyme International Ltd., Wicklow, Ireland) (Mc Cleary 1985). Plant material was treated with 50% ethanol, sodium phosphate buffer (pH 6.5) was added and the samples were incubated in a boiling water bath (100°C). The tubes were equilibrated at 50 °C, treated with lichenase enzyme to cleave the mixture of β-glucans to β-gluco-oligosaccharides (50U/ml; Megazyme International Ireland Ltd) and then further incubated at 50 °C during 60 min. Then, an acetate buffer (pH 4.0) solution was added and the tubes were centrifuged for 10 min, after which aliquots were removed and treated with β–glucosidase (2 U/ml, Megazyme International Ireland Ltd) for another 10 min, to cleave β-gluco-

 

oligosaccharides to glucose. It was noticed that the reaction mixture was incubated with glucose oxidase peroxidase reagent (GOPOD; Megazyme International Ireland Ltd) for 20 min; which thereafter was detected at k = 510 nm using spectrophotometer (MetertechSP-8001 uv/vis spectrophotometer) and retroactively calculated relatively to the content of soluble β-glucan as showed by the formula:

$$\beta - glucan = \Delta E \times (F/W) \times 8.46 \quad (1)$$

where ΔE is the absorbance after β-glucosidase treatment minus blank absorbance; F is the factor for conversion of absorbance values to micrograms quantity of glucose; and W is the weight of sample analysed (Mc Cleary, 1985 ; Mc Cleary and Codd., 1991).

**2.6 Statistical analyses**

Duplicate treatments were assessed. Absorbance data were converted by reference formula into g/100 g of dry weight (DW) for β-glucans. Results are expressed as means ± standard deviations. The ANOVA analysis was carried out for individual and combined across locations. A posteriori multiple comparisons were used and differences among means were located using Duncan's test. The Pearson's simple correlation coefficient r on the data from cultivars and lines set was also computed (Bower, 2009).

**3. Results**

Four accessions of *A. magna* and *A.murphyi* were used in interspecific crosses with different Moroccan cultivars of *A. sativa* (Amlal, Ghali, Soualem, Tissir and Zahri). The selection of the assessed derivative lines for groat β-glucan content was based on the results of the evaluation of their agronomic performance, especially for their good disease tolerance mainly to crown rust and yellow dwarf virus barley protein content, and (Saidi, 2015). Groat β-glucan content assessment of the sixteen derivative hexaploid oat lines in addition to their six hexaploid parents of common oat revealed the existence of variability between the studied lines, related to each experimental location (Tables 2 and 3). When examined in Marchouch location, groat β-glucan content ranged from 1,37±0,09 to 6,05±1,19%, while in the Allal Tazi location, values ranged from 3,06±0,09 to 4,38±0,44%.

In each group of the undertaken crosses (figure 1), some derivative lines exceeded their hexaploid parent for groat β-glucan content. Compared to their tetraploid parents, seven cross combinations among the eight, have yielded lines with high or even higher β-glucan content than their tetraploid parents. Hence, according to the obtained results, the hexaploid parents Ghali, Soualem and Tissir cultivars have shown a great effect noticed for the derivate lines in improving their groat β-glucan content. Therefore, these cultivars are good candidate to be included in future breeding programs aiming the enhancement of groat β-glucan content and therefore develop new lines with good nutritive value which can be conceived for human consumption.

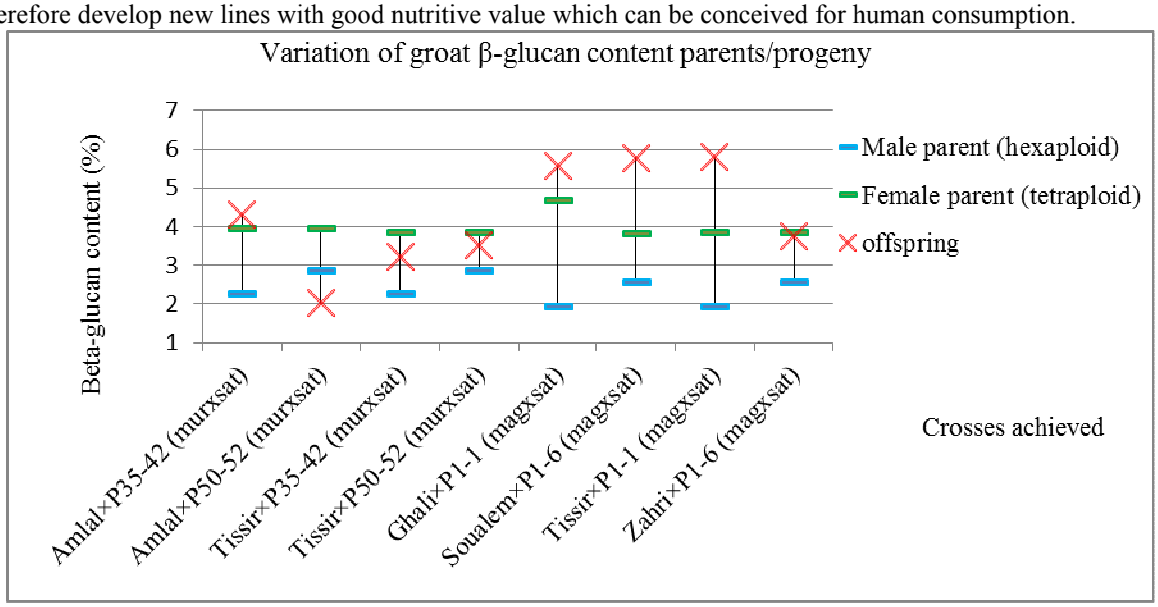


Figure 1. Variation of β-glucan content of the assessed hexaploid oat lines, cultivars and tetraploid accessions

Location 1: Marchouch

Recorded groat β-glucan contents ranged from (1,37±0,09) to (6,05±1,19)% for the line $F_{10\text{-}6}$ (murxsat) and Zahri cultivar, respectively. The average β-glucan content for all tested material was estimated equal to 3,8%. Among the 22 assessed lines / cultivars, 10 had a groat β-glucan content ranging from 3,8% to 6,05%. However, among the hexaploid parents, Zahri cultivar recorded the highest β-glucan value (6,05±1,19)%, followed by Ghali (4,69±0,24) and then Tissir (3,85±0,03) (Table 2).

Location 2: Allal Tazi

 

In Allal Tazi location, unexpected storm happened just before harvest, this has leaded to the loss of some lines and therefore, we managed to harvest seeds only 11 cultivars and lines. Hence, the recorded groat β-glucan content ranged from (3,06±0,09) to (4,38±0,44)% with Zahri and the line $F_{11-7}$ (magxsat) derivative of the cross involving *A.magna*, respectively. The average value for all tested material was of 3,68% (Table 3). Among the 11 assessed lines / cultivars, 6 had a groat β-glucan content ranging from 3,6% to 4,3%.

In the first location, the difference between the lines derivative from the crosses which involved *A. magna* or *A. murphyi* regarding this trait is noteworthy, ranging from 3.44 to 5.79% for *A. magna* derivative lines; and from 1.37 to 4.29% for *A. murphyi* derivative lines, respectively. Therefore, the derivative lines of *A. magna* developed a rich groat β-glucan content compared to that of *A. murphyi*'s derivative lines. As expected in both locations, developed lines and cultivars contain a higher percentage of β-glucan compared to the naked oat cultivar 'Bounejmate' (2,53±0,02%). This is in accordance with what Bhatty (1995) reported that the β-glucans are mainly found in the casing surface of cereal grain layers, and that hulled oats are nutritionally superior compared to conventional naked oats. This may be due to a thin non-lignified husk on the outside of the grain which falls off during harvesting and results in a grain of lower fiber content compared to covered oats.

Table 2. Mean ± standard deviation (SD) of β-glucans levels recorded in the assessed hexaploid lines and cultivars grown in the Marchouch experimental station.

| Oat cultivars / lines | Dry mass content | β-glucan content (g/100g of DW) |
|---|---|---|
| Bounejemate* | 91,59±0,05 | 2,53±0,02 |
| Tissir* | 91,58±0,02 | 3,85±0,03 |
| Zahri* | 91,77±0,14 | 6,05±1,19 |
| Soualem* | 92,10±0,01 | 3,81±0,03 |
| Amlal* | 90,41±0,12 | 2,76±0,21 |
| Ghali* | 91,05±0,02 | 4,69±0,24 |
| $F_{11-1}$† | 91,88±0,07 | 3,44±0,05 |
| $F_{11-2}$† | 89,02±0,32 | 3,66±0,12 |
| $F_{11-3}$† | 91,26±0,21 | 3,95±0,07 |
| $F_{11-4}$† | 87,26±0,39 | 5,77±0,2 |
| $F_{11-5}$† | 89,47±0,12 | 5,79±0,31 |
| $F_{11-7}$† | 92,11±0,04 | 3,75±0,09 |
| $F_{11-8}$† | 90,05±0,05 | 5,55±0,41 |
| $F_{10-1}$‡ | 89,60±0,17 | 4,29±0,26 |
| $F_{10-2}$‡ | 90,65±0,07 | 3,50±0,09 |
| $F_{10-3}$‡ | 90,41±0,23 | 4,07±0,07 |
| $F_{10-4}$‡ | 90,17±0,36 | 2,92±0,04 |
| $F_{10-5}$‡ | 92,35±0,12 | 2,03±0,12 |
| $F_{10-6}$‡ | 92,15±0,05 | 1,37±0,09 |
| $F_{10-7}$‡ | 92,88±0,02 | 3,16±0,26 |
| $F_{10-8}$‡ | 90,62±0,07 | 3,51±0,07 |
| $F_{10-9}$‡ | 93,13±0,05 | 3,21±0,08 |

*varieties
† *(A. sativa x A. magna) x A. sativa*
‡ *(A. sativa x A. murphyi) x A. Sativa*

According to previous studies, oat grain β-glucan contents varies from 3 to 5% (Chernyshova *et al*., 2007 ; Malkki *et al*., 2004) and therefore, our results fits within this range. Nonetheless, some lines were exceptionally distinguishable since their groat β-glucan content exceeded what was previously quoted, as the case for Zahri (6,05±1,19) and the lines $F_{11-4}$ (magxsat) (5,77±0,2)%, $F_{11-5}$ (magxsat) (5,79±0,31)% and $F_{11-8}$ (magxsat) (5,55±0,41)% issued from the crosses with *A. magna*. It was noticed that the comparison of the obtained results in both experimental locations revealed that derivative lines and their hexaploid parent's had a β-glucan content higher than 5% when grown in the Marchouch experimental station.

 

Table 3. Mean ± standard deviation (SD) of β-glucans levels recorded in the assessed hexaploid lines and cultivars grown in the Allal Tazi location.

| Oat cultivars / lines | Dry mass content | β-glucan content (g/100g of DW) |
|---|---|---|
| Tissir* | 96,47±0,19 | 3,63±0,63 |
| Zahri* | 93,86±0,57 | 3,06±0,09 |
| Soualem* | 95,53±0,13 | 4,02±0,07 |
| Amlal* | 95,83±0,33 | 3,28±0,02 |
| $F_{11-1}$† | 95,05±0,17 | 3,74±0,30 |
| $F_{11-2}$† | 96,36±0,13 | 3,58±0,57 |
| $F_{11-3}$† | 90,90±0,14 | 3,76±0,05 |
| $F_{11-5}$† | 90,60±0,41 | 3,92±0,12 |
| $F_{11-7}$† | 95,79±0,28 | 4,38±0,44 |
| $F_{10-3}$‡ | 95,66±0,45 | 3,59±0,12 |
| $F_{10-8}$‡ | 96,05±0,15 | 3,54±0,02 |

*varieties
† *(A. sativa x A. magna) x A. sativa*
‡ *(A. sativa x A. magna) x A. sativa*

Furthermore, there was a highly significant variation (P<0.001) in β-glucan contents, within and between locations, of the assessed material under two different environmental conditions.

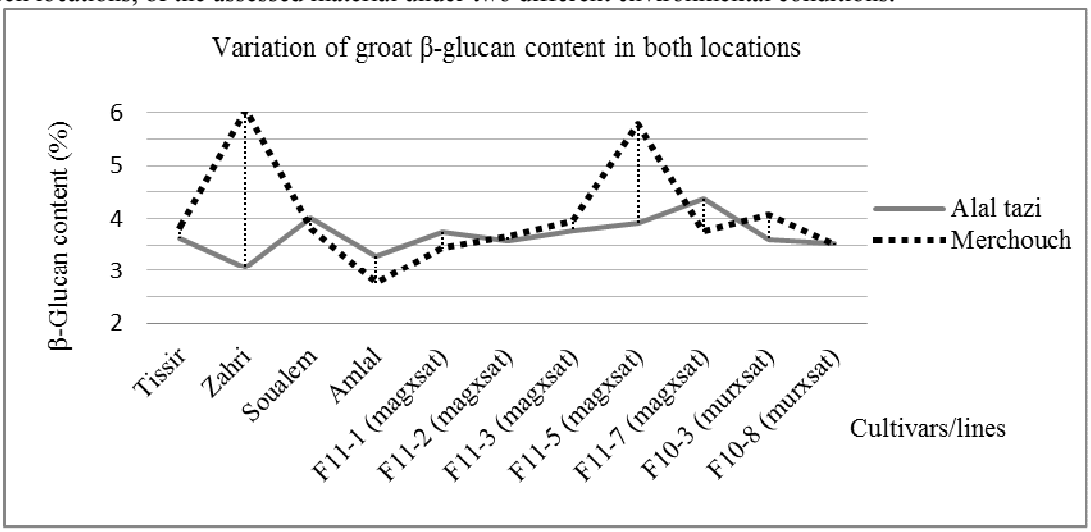


Figure 2. Variation of the groat β-glucan content of the assessed hexaploid oat lines and cultivars grown in the Marchouch and Allal Tazi locations.

As we can notice from the obtained values from both locations (Marchouch and Allal Tazi), the assessed lines and cultivars differed widely considering the location (Table 4 and Figure. 2). Thus, the current study confirmed the results obtained by Zhang *et al*. (2002), where β-glucan content of the same plant material differs according to a targeted environment. But, this hypothesis needs to be confirmed by further research.

As shown in Table 4, the ANOVA analysis for groat β-glucan content of 22 oat cultivars and lines, grown in these two Moroccan locations (Table 4), showed that the influence of genotype, location and their interaction were highly significant ($p < 0.0001$). In comparison, groat β-glucan contents were more influenced by genotype than by environment effect. Nonetheless, it is obvious that their interaction is highly significant, suggesting the importance of selecting for a targeted region (Zhang *et al*., 2002).

 

Table 4. Analysis of variance of β-glucan contents for 22 assessed hexaploid lines and cultivars grown in Marchouch and Allal Tazi locations.

| Source of variation | Degrees of Freedom | Sum of Squares | Mean Square | F value |
|---|---|---|---|---|
| Total | 65 | 67,353 | | |
| Cultivar/line | 21 | 52,637 | 2,507 | 26,22*** |
| Location | 1 | 3,218 | 3,218 | 33,66*** |
| Interaction | 10 | 11,346 | 1,135 | 11,87*** |
| Error | 33 | 3,155 | 0,096 | |

*** : Significant at the 0.001 probability level.

**4. Discussion**

The effects of genotype, environment and their interaction on groat β-glucan content concentration have been reported by previous studies but with inconclusive results (Ajithkumar *et al*., 2005 ; Martinez *et al*., 2010 ; Welch *et al*., 2000 ; Ehrenbergerova *et al*., 2008 ; Redaelli *et al*., 2013 and Demirbas, 2005). Therefore, our results are similar to those reported by several authors such as Welch *et al*. (2000), Ehrenbergerova *et al*. (2008) and Redaelli *et al*. (2013), whom concluded that variation corresponding to groat β-glucan concentration between oat cultivars may be mainly controlled by environmental factors rather than genotype. It is in partial agreement with the findings of Martinez *et al*. (2010) where β-glucan contents were about equally influenced by genotype and environmental effects. This observation is in contradiction when compared to the results obtained by Demirbas (2005) who concluded that the effects of the environmental factors on groat β-glucan content are not significant.

The relationship between β-glucan content and some important agronomic traits, such as irrigation, soil nitrogen level, soil type, etc., must be taken into consideration in order to sort out some of conflicting reports by intensifying and deepening researches.

Important other studies have revealed that selection for high groat β-glucan content will probably give information on its molecular weight and therefore reported the existence of an important positive correlation between groat β-glucan content and its molecular weight (Andersson and Borjesdotter, 2011). It seems that high molecular weight is important for β-glucan's health effects, especially its cholesterol lowering impact (Wolever *et al*., 2010). It is suggested as the result of the conformational variation between the polymers, caused by the ratio and numbers of (1-3)- and (1-4)- β-glycosidic bonds (Andersson and Borjesdotter, 2011). The molecular and structural features are important determinants of β-glucan's physiological properties. So that, the glucose, insulin and cholesterol lowering effects of β-glucan are correlated with the concentration and molecular weight of the solubilised β-glucans in the gastro-intestinal tract, and its ability to increase luminal viscosity (Lazaridou and Biliaderis, 2007 ; Anttila *et al*., 2008). Cereal water-soluble fibers with certain structural features may display determinants functionalities in technological, nutritional and physiolgical traits, attempt to its incorporation in various products and increase its economic value.

Selection of oat cultivars and lines was based upon their agronomic performance in addition to their improved nutritional value through hybridization work. In addition, we should know the type of the acting genes for a given trait, there action and mode of inheritance as well as their heritability (Campbell and Frey, 1972). According to our previous study concerning the assessment of the same plant material for some technological parameters (Manzali *et al*., 2014), the undertaking of the interspecific crosses between hexaploid cultivars of A. sativa and the wild tetraploid oat species *A. magna* and / or *A. murphyi* (Saidi, 2015) has helped in extending their genetic basis by improving the derivative lines's groat protein contents (to 17%), groat oil content (to 10%) and groat β-gucan (to 6%) (Manzali *et al*., 2014). According to the obtained results, in each group of the progenies, some derivative lines exceeded their hexaploid parents for groat β-glucan concentration. This may be due to the association of two factors, good environmental conditions and genetic compatibility between the wild tetraploid accessions and the Moroccan hexaploid cultivars, which is more advantageous in certain combinations. Buerstmayr *et al*. (2007) stated that high genetic diversity within parental cultivar could result in higher degree of heterosis, trangressive segregants and act as buffer against performance reduction due to biotic and abiotic stresses. On this basis, we can assess plant material on different ecological preference and response to environmental fluctuations and novel stress factors among different genotypes (Doring *et al*., 2011).

The results yielded by this study are interesting because the increasing demand for high nutritive value oat cultivars can be satisfied through the new developed oat lines, having high groat β-glucan content. Selected lines can be a source of valuable nutrients for human consumption and therefore can be used for the production of functional foods differing from that commonly used up to now. Thus, the development of new oat cultivars with greater groat β-glucan contents, conceived for human consumption, can help increasing the social economic

 

impact of oat cultivation mainly in North Africa, where oat is still used for animal feed.

## 5. Conclusion

The assessment of groat β-glucan content of some Moroccan hexaploid oat cultivars compared to the newly developed hexaploid improved lines has shown that groat β-glucan content was significantly affected, in one hand, by genotype and, in the other hand, by environmental conditions in addition to the interaction genotype x environment. Certain derivative lines such as $F_{11-4}$ (magxsat), $F_{11-5}$ (magxsat) and $F_{11-8}$ (magxsat), according to their high groat β-glucan content, would be taken into consideration to be incorporated in healthy food formulation. It is noticed that groat β-glucan possesses more important properties as other soluble dietary fibers. However, the nutritional impact of β-glucan has not been fully evaluated and warrant further investigation. Additionally, further research studies need to be carried out in order to optimize β-glucan's dose and to investigate the effect of β-glucan supplementation on human health (as blood lipid chemistry). The eventual goal would be to combine β-glucan supplementation with other dietary means of controlling obesity for example, and to consequently prevent the need for drugs and/or other synthetic products against this disease which becomes popular and considered as a great health problem for many populations around the world.

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