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RAPD ANALYSIS OF THE GENETIC POLYMORPHISM IN EUROPEAN WHEAT GENOTYPES

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ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the main crops for human nutrition. The genetic variability of grown wheat has been reduced by modern agronomic practices, which inturn prompted the importance of search for species that could be useful as a genepool for the improving of flour quality for human consumption or for other industrial uses. Therefore, the aim of this study was to analyze the genetic diversity among 24 European wheat genotypes based on Random Amplified Polymorphism (RAPD) markers. A total of 29 DNA fragments were amplified with an average 4.83 polymorphic fragments per primer. The primer producing the most polymorphic fragments was SIGMA-D-P, where 7 polymorphic amplification products were detected. The lowest number of amplified fragments (3) was detected by using the primer OPB-08. The size of amplified products varied between 300 bp (OPE-07) to 3000 bp (SIGMA-D-P). The diversity index (DI) of the applied RAPD markers ranged from 0.528 (OPB-07) to 0.809 (SIGMA-D-P) with an average of 0.721. The polymorphism information content (PIC) of the markers varied from 0.469 (OPB-07) to 0.798 (SIGMA-D-P) with an average 0.692. Probability of identity (PI) was low ranged from 0.009 (SIGMA-D-P) to 0.165 (OPB-07) with an avarage 0.043. The dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared. Within the dendrogram was separated the unique genotype Insegrain (FRA) from the rest of 23 genotypes which were further subdivided into two subclusters. In the first subclaster were grouped 13 genotypes and the second subcluster involved 10 genotypes. The first subcluster also included the genotype Bagou from France, in which were detected novel high – molecular – weight glutenin subunits using SDS-PAGE. Using 6 RAPD markers only two wheat genotypes have not been distinguished. Through that the information about genetic similarity and differences will be helpful to avoid any possibility of elite germplasm becoming genetically uniform.

Keywords: *Triticum aestivum* L.; PCR; RAPD marker; genetic diversity

INTRODUCTION

Wheat (*Triticum* spp.) is a self-pollinating annual plant, belonging to the family *Poaceae* (grasses), tribe *Triticeae*, genus *Triticum*. According to different classifications, number of species in the genus varies from 5 to 27. The two main groups of commercial wheats are the durums (*Triticum durum* L.) and bread wheats (*Triticum aestivum* L.) with 28 and 42 chromosomes respectively **(Šramková et al., 2009)**.

Bread wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated crops used mainly for human consumption and support in the world.

The importance of wheat is mainly due to the fact that its seed can be ground into flour, which form the basic ingredients of bread and other bakery products, as well as pastas, and thus it presents the main source of nutrients such as proteins, carbohydrates, lipids, fibre and vitamins, to the most of the world population. Agronomical and nutritionally important status of wheat among the several other cereal crops has obtained because of its large genome size and multifaceted uses. Approximately 734.8 million tons of wheat is produced annually on 247 million hectare of the total cultivated land in the world and supports nearly 35% of the world´s population (http://www.fao.org/worldfoodsituation/csdb/en/).

Enormously growing population and the changing of life style have posed challenges to the wheat breeders to develop newer wheat varieties with high yielding performance, high quality seed and resistance to pests and stress conditions. Modern agronomic practices have reduced the genetic variability of cultivated wheats, which has given great importance in the search for could be useful in contributing genes for wheat improvement **(Jauhar, 1993)**.

Characterization of genetic diversity and genetic relatedness is a fundamental element in crop improvement strategies **(Zhu et al., 2000)**. Like any other crops, the first step of in wheat improvement is full assessment of the local materials, including collection, evaluation and molecular characterization of germplasm lines. Knowledge about morphological and agronomic traits and genetic relationships among breeding materials could be an invaluable aid in crop improvement strategies **(Abbas et al., 2008)**.

A number of methods are currently used for analysis of genetic diversity in germplasm accessions, breeding lines

and segregating populations. These methods were based on pedigree, morphological, agronomic performance, biochemical and molecular (DNA-based) data **(Mohammadi and Prasanna, 2003)**. The diversity patterns allow plant breeders to better understand the evolutionary relationships among accessions and to incorporate useful genotypes in the breeding programs **(Thompson et al., 1998)**.

However, diversity estimates based on pedigree analysis have generally been found inflated and unrealistic **(Fufa et al., 2005)**. Genetic diversity estimates based on morphological traits, on the other hand, suffer from the drawback that such traits are limited in number and are influenced by environment **(Maric et al., 2004)**. Molecular markers are useful tools for estimating genetic diversity as these are not influenced by environment, are abundant and do not require previous pedigree information. Among the biochemical markers, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS – PAGE) has been widely used due to its simplicity and effectiveness for estimating genetic diversity.

Among the various DNA – based markers, AFLP (Amplified Fragment Length Polymorphism) and RFLP (Restriction Fragment Length Polymorphism) have been used to study genetic diversity. These markers elucidate the phylogenetic relationships among various lines, for their efficient use in breeding and genetic resource management. These methods, however, involve the use of expensive enzymes, radioactive labeling, and are cumbersome and hence, appear unsuitable **(Gajera et al., 2010)**.

On the other hand, RAPD (Random Amplified Polymorphic DNA) markers have offered a valuable opportunity to characterize genetic variation and structure in plant population **(Ayana et al., 2000)** with requiring only small amounts of DNA sample without involving radioactive labels, are simplier as well as faster, and therefore they have been increasingly employed for analysis of genetic diversity **(Ebrahimi et al., 2011)**.

The use of RAPD molecular markers are routine methods for quickly and efficiently estimating relationships between lines and populations of many plant species. It is assumed that these markers are randomly spaced throughout the genome **(Mark et al., 1999)**. RAPD markers have proven to be quite efficient in detecting genetic variations and used for diversity assessment and for identifying germplasm in a number of plant species such as wheat **(Ahmed et al., 2010**; **Mahmood et al., 2011**; **Cifci and Yagdi, 2012**; **Rehman et al., 2013)**, rye **(Petrovičová et al., 2014)**, flax **(Bežo et al., 2005)**, castor **(Vivodík at al., 2015)**, amaranth **(Štefúnová et al., 2013)** or Echinacea **(Kapteyn et al., 2002)**, and also these markers have been used for identification and differentiation on other microbial or animal level, such as in the yeast microbiota grown on grapes **(Drozdz et al., 2015)** or in fishery food products **(Bajzík et al., 2010)**.

The present study is focused on estimation of genetic distance between 24 European wheat genotypes, included the genotype with probably novel high – molecular – weight glutenin subunits identified by SDS – PAGE **(Kuťka Hlozáková et al., 2015)** based on 6 RAPD markers. Although the information gathered here would be helpful in future for genomic mapping studies leading to development of wheat cultivars with broader genetic background to obtain improved crop productivity.

MATERIAL AND METHODOLOGY

Plant material: Twenty – four genotypes of hexaploid wheat (*Triticum aestivum* L.) grain originating from five different geographical areas (Slovakia, Czech Republic, Hungary, Germany and France) of Europe were obtained from the collection of genetic wheat resources of the Gene Bank of Slovak Republic in Piešťany.

Genomic DNA Isolation: DNA of 24 genotypes of wheat was extracted from the endosperm of intact, dry and mature single seeds using the Gene JET Plant Genomic DNA Purification Mini Kit (Thermo Scientific) supplemented with 2% polyvinylpyrrolidone (PVP) in lysis buffer.

RAPD Analysis: Amplification of RAPD fragments was performed according to **Cifci and Yagdi (2012)** using decamer arbitrary primers (Operon technologies Inc, USA; SIGMAD, USA). Amplifications were performed in a 25 µL reaction volume containing 5 µL DNA (100 ng), 12.5 µL Master Mix (Promega), and 1 µL of 10 pmol of primer. Amplification was performed in a programmed thermocycler (Biometra, Germany) with initial denaturation at 94 °C for 3 min., 40 cycles of denaturation at 94 °C for 30 sec., primer annealing at 38 °C for 1 min., extension at 72 °C for 2 min., and final extension at 72 °C for 10 min. Amplified products were separated on 1.2% agarose in 1 × TBE buffer. The gels were stained with ethidium bromide, visualised under ultraviolet (UV) light and documented using gel documentation system Grab-It 1D for Windows. The molecular weight of amplified fragments was estimated with the help of Thermo Scientific FastRuler Middle Range DNA Ladder (MBI, Fermentas).

Data analysis: The RAPD bands were scored as present (1) or absent (0), each of which was treated as an independent character regardless of its intensity. The binary data generated were used to estimate levels of polymorphism by dividing the polymorphic bands by the total number of scored bands and to prepare a dendrogram. A dendrogram based on hierarchical cluster analysis using the unweighted pair group method with arithmetic average (UPGMA) with the software package SPSS professional statistics version 17 was constructed. For the assessment of the polymorphism in the wheat genotypes using RAPD markers in their differentiation we used diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau et al., 1995)** and polymotphic information content (PIC) **(Weber, 1990)**, which were calculating according to formulas:

Diversity index (DI)

$$DI = 1 - \sum p_i^2$$

Probability of identity (PI)

$$PI = \sum p_i^4 + \sum_{i=1}^{i=n-1} \sum_{j=i+1}^{n} \left(2 p_i p_j\right)^2$$

information content (PIC)

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2 \right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 \cdot p_j^2$$

(where p_i and p_j are frequencies of i^{th} and j^{th} fragment of given genotypes)

RESULTS AND DISCUSSION

Efficient and effective crop improvement program depends on the extent of genetic diversity either existing or created. The breeding of wheat has achieved hallmark progress that is able to feed ever increasing population in the world **(Rehman et al., 2013)**. Genetic diversity is one of the key factors for the improvement of many crop plants including wheat **(Ahmed et al., 2010)**. The efficiency of genetic gain by selection can be improved if the patterns of genetic diversity within a population of breeding lines are known. Genetic similarity or distance estimates among genotypes are helpful in the selection of parents to be used in the breeding program **(Van Becelaere et al., 2005)**.

In this work, 6 primers were screened for PCR amplification of DNA and RAPD analysis in 24 wheat genotypes. Table 1 shows codes and sequences of these primers, total number of amplified fragments from 24 wheat genotypes, the diversity index, the polymorphic information content and the probability of identity for each primer. All the primers produced 29 DNA fragments (Figure 1) with an average of 4.833 bands per primer. From these six primers, primer SIGMA-D-P was the most polymorphic, where 7 polymorphic amplification products were detected. The lowest number of different fragments (3) was detected in primer OPB-08. Of the 29 amplified bands, all 29 were polymorphic, with an average of 4.83 polymorphic bands per primer. The size of amplified products varied from 300 bp (OPE-07) to 3000 bp (SIGMA-D-P).

To determine the level of polymorphism in the analysed group of wheat genotypes, diversity index DI, probability of identity PI and polymorphic information content PIC were calculated. All three indicators were applied for all six RAPD primers and for their calculation, the individual frequences of fragments of each marker were used.

The diversity index (DI) of the applied RAPD markers ranged from 0.528 (OPB-07) to 0.809 (SIGMA-D-P) with

Table 1 List of RAPD primers, total number of bands and the statistical characteristics og the used RAPD markers.

Primers	Primer sequence (5´-3´)	Total number of bands	DI	PIC	PI
OPA-02	TGCCGAGCTG	5	0.761	0.736	0.016
OPA-03	AGTCAGCCAC	5	0.741	0.712	0.023
OPA-13	CAGCACCCAC	4	0.708	0.668	0.033
OPB-08	GTCCACACGG	3	0.528	0.469	0.165
OPE-07	AGATGCAGCC	5	0.779	0.768	0.014
SIGMA-D-P	TGGACCGGTG	7	0.809	0.798	0.009
Total	-	**29**	-	-	-
Avarage	-	**4.833**	**0.721**	**0.692**	**0.043**

Note: DI – diversity index, PIC – polymorphic information content, PI – probability of identity.

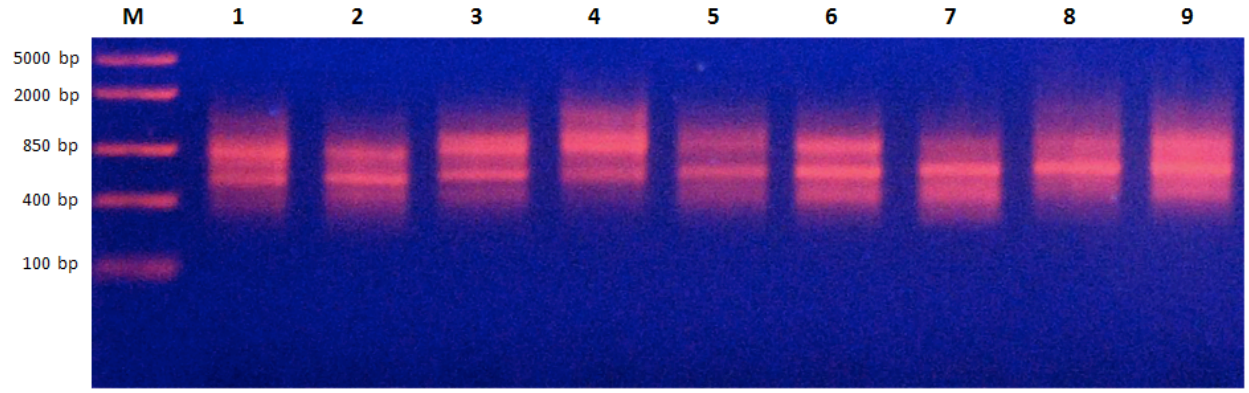

Figure 1 PCR amplification products of nine wheat genotypes with SIGMA-D-P primer: Lane M – Thermo Scientific FastRuler Middle Range DNA Ladder, 1 – Banquet (CZE), 2 – Kalif (FRA), 3 – Bonpain (FRA), 4 – Verita (SVK), 5 – Hana (CZE), 6 – MV Optima (HUN), 7 – Balthasar (FRA), 8 – Bagou (FRA), 9 – Ilona (SVK).

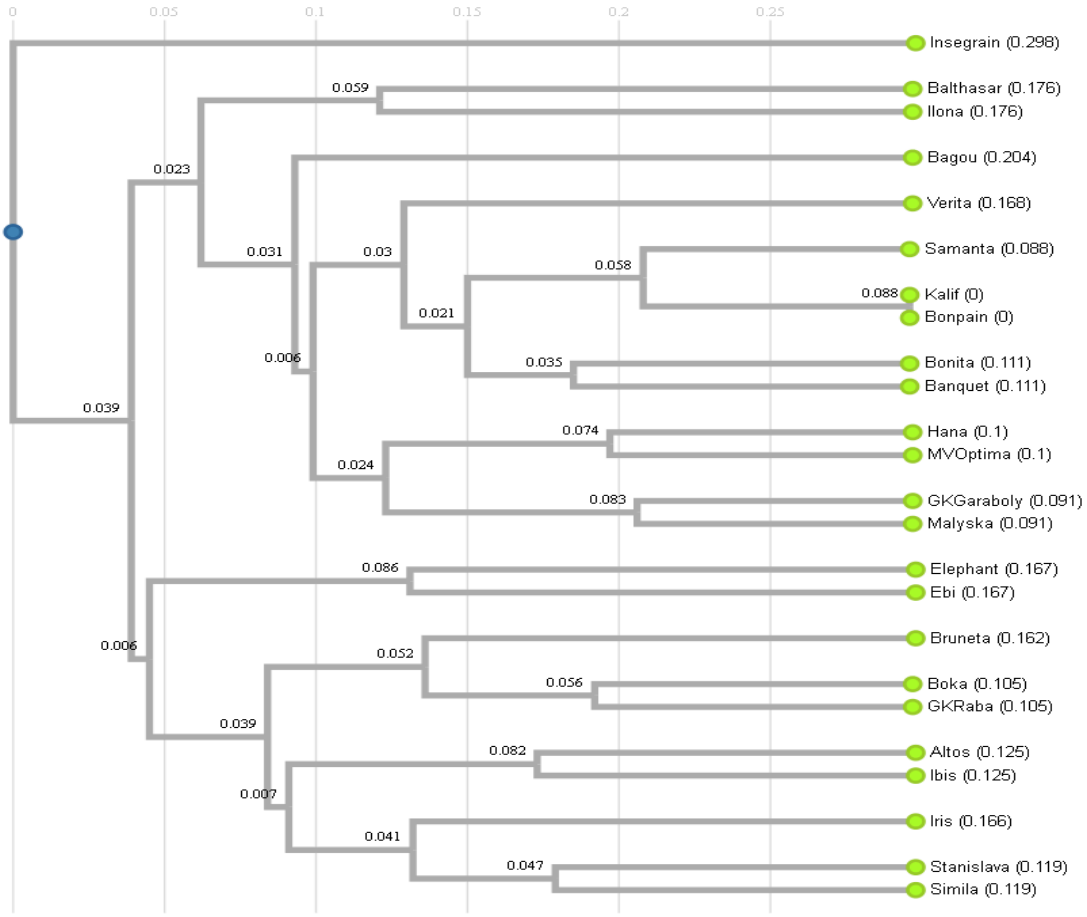

Figure 2 Dendrogram based on 29 RAPD fragments in 24 wheat genotypes.

an avarage of 0.721. The polymorphism information content (PIC) of the markers varied from 0.469 (OPB-07) to 0.798 (SIGMA-D-P) with an average 0.692. 83% of used RAPD markers had PIC and DI values higher than 0.6 that means high polymorphism of chosen markers used for analysis **(Vivodík et al., 2015)**. Probability of identity (PI) was ranged from 0.009 (SIGMA-D-P) to 0.165 (OPB-07) with an average 0.043. Cause of that, it is necessary to use a higher number of RAPD markers.

For the detection of genetic diversity, the dendrogram based on hierarchical cluster analysis using UPGMA algorithm was prepared (Figure 2). This dendrogram separated unique genotype Insegrain (FRA, cluster I) from other 23 genotypes (cluster II) that were further subdivided into 2 subclusters. In the first subcluster were grouped 13 genotypes from Slovakia (38.5%), France (30.8%), Czech Republic (15.4%) and the same number (15.4%) from Hungary. Genotypes Kalif and Bonpain from France could not be distinguished because of their close genetic similarity. This subcluster also included the genotype Bagou from France, in which were detected novel high – molecular – weight glutenin subunits using SDS – PAGE. The second subcluster involved 10 genotypes from Slovakia (30%), Czech Republic (30%), Germany (20%), France (10%) and the same number (10%) from Hungary.

Lower polymorphism using RAPD analysis was detected by **Cifci and Yagdi (2012)** who used 17 markers to describe genetic similarity of 16 Turkish wheat genotypes. PIC values ranged from 0.11 to 0.92 with an average 0.59. **Ahmed et al., (2010)** also used 15 RAPD markers to analyse the genetic diversity of 32 wheat breeding lines and reached an average 4.1% polymorphism per primer.

On the other hand, high polymorphism was detected in set of amaranth **(Štefúnová et al., 2013)**, rye **(Petrovičová et al., 2014)** or castor genotypes **(Vivodík et al., 2015)**. Also higher polymorphism for RAPD was detected in Pakistan wheat landraces, where **Mahmood et al., (2011)** reached an average 7.8 % polymorphism per primer using 10 RAPD markers.

CONCLUSION

The present study was aimed to determine the genetic variation among wheat genotypes grown in Europe. Our results showed that RAPD markers are useful for exploring genetic diversity of raw material for developing new varieties. The dendrogram prepared based on UPGMA algorithm separated the unique genotype Insegrain (FRA) from the rest of 23 genotypes which were further subdivided into two subclusters. The first subcluster also included the genotype Bagou from France, in which were detected novel high – molecular – weight glutenin subunits using SDS-PAGE. Only two wheat genotypes have not been distinguished using these 6 RAPD markers. For better resolution of the analysed wheat genotypes, it is necessary to use a higher number of RAPD markers.

Despite that, the information gathered here would be helpful in genomic mapping studies and for the development of wheat cultivars with wider and diverse genetic background to obtain improved crop productivity.

REFERENCES

Abbas, S. J., Shah, S. R. U., Rasool, G., Iqbal, A. 2008. Analysis of genetic diversity in Pakistani wheat varieties by using randomly amplified polymorphic DNA (RAPD) primers. *American-Eurasian Journal of Sustainable Agriculture*, vol. 2, no. 1, p. 29-33.

Ahmed, F. A., Iqbal, M., Masood, M. S., Rabbani, M. A., Munir, M. 2010. Assessment of genetic diversity among Pakistani wheat (*Triticum aestivum* L.) advanced breeding lines using RAPD and SDS – PAGE. *Electronic Journal of Biotechnology*, vol. 13, no. 3 (online). http://dx.doi.org/10.2225/vol13-issue3-fulltext-2

Ayana, A., Bryngelsson, T., Bekele, E. 2000. Genetic Variation in Wild Sorghum (Sorghum Bicolor Ssp. Verticilliflorum (L.) Moench) Germplasm from Ethiopia Assessed by Random Amplified Polymorphic DNA (RAPD). *Genetic Resources and Crop Evolution*, vol. 47, no. 1, p. 471-482. http://dx.doi.org/10.1111/j.1601-5223.2000.t01-100249.x

Bajzík, P., Golian, J., Židek, R., Čapla, J., Belej, Ľ., Ondrejka, M., Mrázová, Ľ., Maršálková, L. 2010. Methods for fish species identification in food products. *Potravinarstvo*, vol. 4, no.3, p. 1-5. http://dx.doi.org/10.5219/25

Bežo, M., Candráková, A., Štefúnová, V., Žiarovská, J., Ražná, K., 2005. Identification of flax germplasm collection duplicates using randomly amplified polymorphism DNA markers (RAPD). *Acta fytotechnica et zootechnica*, vol. 8, no. 3, p. 62-66.

Cifci, E. A., Yagdi, K. 2012. Study of genetic diversity in wheat (*Triticum aestivum*) varieties using random amplified polymorphic DNA (RAPD) analysis. *Turkish Journal of Field Crop*, vol. 17, no. 1, p. 91-95.

Drozdz, I., Makarewicz, M., Sroka, P., Satora, O., Jankowski, P. 2015. Comparison of the yeast microbiota of different varietis of cool-climate grapes by PCR-RAPD. *Potravinarstvo*, vol. 9, no. 1, p. 293-298. http://dx.doi.org/10.5219/484

Ebrahimi, F., Mohammadi-Nejad, G., Baghizadeh, A., Abdolinejad, M. 2011. Genetic diversity evaluation of rapeseed genotypes (Brassica napus L.) based on phenotypic traits and random amplified polymorphic DNA (RAPD) markers. *African Journal of Biotechnology*, vol. 10, no. 76, p. 17391-17398. http://dx.doi.org/10.5897/ajb11.1786

Fufa, H., Baenziger, P. S., Beecher, B. S. M, Dweikat, I., Graybosh, R. A., Eskridge, K. M. 2005. Comparison of phenotipic and molecular marker – based classifications of hard red winter wheat cultivars. *Euphytica*, vol. 145, no. 1-2, p. 133-146. http://dx.doi.org/10.1007/s10681-005-0626-3

Gajera, B. B., Kumar, N., Singh, A. S., Punvar, B. S., Ravikiran, R., Subhash, N., Jadeja, G. C. 2010. Assessment of genetic diversity in castor (*Ricinus communis* L.) using RAPD and ISSR markers. *Industrial Crops and Products*, vol. 32, no. 3, p. 491-498. http://dx.doi.org/10.1016/j.indcrop.2010.06.021

Jauhar. P. P. 1993. Alien gene transfer and genetic enrichment of bread wheat. Damania AB (ed) *Biodiversity and wheat improvement*, p. 103-119. ISBN 0-471-94137-9

Kapteyn, J., Simon, J. E. 2002. The use of RAPDs for assessment of identity, diversity and quality of Echinacea. *Janick, J., Whipkey, A. (Eds.), Trends in New Crops and New Uses.* ASHS Press, Alexandira, VA, p. 509-513.

Kuťka Hlozáková, T., Gregová, E., Gálová, Z. 2015. Genetic diversity of Glu – 1 in European wheat genetic resources and varieties. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. 2, p. 23-25. http://dx.doi.org/10.15414/jmbfs.2015.4.special2.23-25

Mahmood, T., Siddiqua, A., Rasheed, A., Nazar, N. 2011. Evaluation of genetic diversity in different Pakistani wheat land races. *Pakistan Journal of Bot*any, vol. 43, no. 2, p. 1233-1239. http://dx.doi.org/10.3923/pjbs.2006.2858.2861

Maric, S., Bolaric, S., Martincic, J., Pejic, I., Kozumplink, V. 2004. Genetic diversity of hexaploid wheat cultivars estimated by RAPD markers, morphological traits and coefficients of parentage. *Plant Breeding*, vol. 123, no. 4, p. 366-369. http://dx.doi.org/10.1111/j.1439-0523.2004.00956.x

Mark, S., Adam, B., Lukaszewski, J., Whitkus, R. 1999. Development of arm specific RAPD markers for rye chromosome 2R in wheat. *Crop Science*, vol. 39, no. 6, p. 1702-1706. http://dx.doi.org/10.2135/cropsci1999.3961702x

Mohammadi, S. A., Prasanna, B. M. 2003. Analysis of genetic diversity in crop plant – salient statistical tools and consideration: Review and interpretation. *Crop Science*, vol. 43, no. 4, p. 1235-1248. http://dx.doi.org/10.2135/cropsci2003.1235

Paetkau, D., Calvert, W., Stirling I., Strobeck, C. 1995. Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, vol. 4, no. 3, p. 347-354. http://dx.doi.org/10.1111/j.1365-294x.1995.tb00227.x

Petrovičová, L., Gálová, Z., Balážová, Ž., Vivodík, M., Wójcik-Jagla, M., Rapacz, M., 2014. *Genetic diversity of czechoslovak origin rye cultivars detected by RAPD markers. CECE 2014*. Brno: Institute of analytical chemistry AS CR, p. 353-356. ISBN 978-80-904959-2-0.

Rehman, R., Shah, K. N., Masood, M. S., Arshad, M., Ghafoor, A. 2013. Genetic divergence among Pakistani bread wheat varieties and advanced lines for randomly amplified polymorphic DNA (RAPD) markers. *Pakistan Journal of Botany*, vol. 45, no. S1, p. 327-332.

Šramková, Z., Gregová, E., Šturdík, E. 2009. Genetic improvement of wheat. *Nova Biotechnologica*, vol. 9, no. 1, p. 27-51.

Štefúnová, V., Bežo, M., Labajová, M. 2013. Molecular identification of amaranth (Amaranthus L.) species used in nutrition. *Potravinarstv*o, vol. 7, special no., p. 231-234. http://www.potravinarstvo.com/dokumenty/mc_march_2013/bezpecnost_potravin_rastlinneho_povodu/stefunova.pdf

Thompson, J. A., Nelson, R. L., Vodkin, L. O. 1998. Identification of diverse soybean germplasm using RAPD markers. *Crop Science*, vol. 38, no. 5, p. 1348-1355. http://dx.doi.org/10.2135/cropsci1998.0011183x003800050033x

Van Becelaere, G., Lumbbers, E. L., Paterson, A. H., Chee, P. W. 2005. Pedigree vs. DNA marker – based genetic similarity estimates in cotton. *Crop Science*, vol. 45, no. 6, p. 2281-2287. http://dx.doi.org/10.2135/cropsci2004.0715

Vivodík, M., Balážová, Ž., Gálová, Z., Kuťka Hlozáková, T. 2015. Differentiation of ricin using RAPD markers. *Pakistan Journal of Botany*, vol. 47, no. 4, p. 1341-1345.

Weber, J. L. 1990. Informativeness of human (dC-dA)n x (dG-dT)n polymorphism. *Genomics*, vol. 7, no. 4, p. 524-530. http://dx.doi.org/10.1016/0888-7543(90)90195-z

Weir, B. S. 1990. Genetic data analysis. Methods for discrete population genetic data. http://dx.doi.org/10.2307/2532683

Zhu, Y., Chen, H., Fan, W., Wanf, Y., Li, Y., Chen, J., Yang, S., Hu, L., Leung, H., Mew, T. W., Teng, P. S., Wang, Z., Mundit, C. C. 2000. Genetic diversity and disease control in rice. *Nature*, vol. 406, p. 718-722. http://dx.doi:10.1038/35021046

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