Development of SCAR markers for molecular tagging of drought tolerance QTL in barley

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Abstract: Two sequence characterized amplified region (SCAR) markers were developed from two randomly amplified polymorphic DNA (RAPD) in barley. E9 [CTTCACCCGA] and A19 [CAAACGTCGG] are RAPD markers produced two fragments that were proved to be linked to drought tolerant traits (relative water content (RWC), osmotic potential (OP), number of leaves on the main stem (NL)). The two fragments were isolated, cloned, sequenced, and converted into SCAR markers (SCE9_600 and SCA19_800). Testing designed SCAR primers were performed using RIL population derived from a cross between 'Tadmor' (drought tolerant) and 'Er/Apm' (drought susceptible) parents. Both SCAR markers followed the Mendelian inheritance of segregation. However, only SCA19_800 marker was mapped to linkage group number 1. The amplified fragment from E9 RAPD primer showed 95% homology with CBF12 gene in *Hordeum vulgare* subsp. vulgare retrotransposon associated with levels of freezing tolerance in temperate-climate cereals. This is the first attempt of SCAR markers development from RAPD markers linked to the drought tolerant traits (RWC, OP, NL) in barley. The development of reproducible markers such as SCAR is essential to facilitate their use in barley breeding programs.
[Muhanad Walid Akash. **Development of SCAR markers for molecular tagging of drought tolerance QTL in barley.** *Life Sci J* 2013;10(12s):1056-1060] (ISSN: 1097-8135). http://www.lifesciencesite.com. 168

Keywords: SCAR, Barley, Drought, QTL analysis

1- Introduction

Drought is one of the most important abiotic factors constraining barley production, causing complete grain failure in severely affected fields. Good relative water content (RWC) (Matin, et al., 1989) and high osmotic potential (OP) (Blum, 1989) are two important traits that add to plant ability to tolerate drought conditions in barley. Also black kernel color was found to be correlated with drought tolerant in barley (Yasseen and Al-Maamari, 1995). Results of Barley QTL studies revealed two main random amplified polymorphic DNA (RAPD) markers that are linked to RWC, OP and number of leaves on the main stem (NL) (Teulat et al., 1997; Teulat et al., 1998; Teulat et al., 2001). These studies used RIL population derived from a cross between 'Tadmor' (drought tolerant) and 'Er/Apm' (drought susceptible) parents. Several type of markers were found to be tightly linked to RWA and OP, of which, two RAPD markers (E9 [CTTCACCCGA] and A19 [CAAACGTCGG]) were detected. E9 has been found to be tightly linked in coupling with OP at full turgor with 2.26 LOD, 0.06 estimated additive effect and 17.1% explained variation (Teulat et al., 2001). E9 has also been found to be tightly linked with RWC with 2.01 LOD, 0.07 estimated genetic additive effect and 10.8% explained variation (Teulat et al., 1998). A19 has been found to be tightly linked with (NL) with 3.51 LOD, 0.44 estimated genetic effect and 27.1% explained variation (Teulat et al., 1997).

RAPD marker procedure is simple, fast, does not need previous sequence information, and usually amplify several genetic loci. However, RAPD markers are sensitive to reaction conditions which result in low reproducibility and hinder its uses and applications. For this reason it is important to transform RAPD into highly reproducible marker, called sequence characterized amplified region (SCAR). RAPD can be transformed into SCAR marker by cloning and sequencing the two ends of the amplified products and synthesizing two longer primers homologous to each end (Paran and Michelmore, 1993). With a highly stringent annealing temperature, SCAR marker is less sensitive to reaction condition and resulted in a reproducible amplification of single loci. By being both accurate and cost efficient, SCAR markers offer the most practical method for screening numerous samples in a time and labor-saving manner (Kasai et al., 2000). SCAR marker can be easily employed in molecular breeding programs such as marker assisted selection for drought tolerant. In barley, SCAR markers have been developed for many traits (Deng, et al., 1997; Eckstein, 2002; Hernandez et al., 1999; Ardiel, et al., 2002; Genger et al. 2003). The objective of this study was to develop a SCAR marker linked to RWC, OP and NL QTL in barley.

2- Materials and Methods

Plant material

Two RAPD markers (E9 [CTTCACCCGA] and A19 [CAAACGTCGG]) linked to RWC, OP, NL were previously ;detected using QTL analysis (Teulat et al., 1997; Teulat et al., 1998; Teulat et al., 2001).

Development of RIL population for the QTL analysis has been described previously by Akash, (2010). In short, a barley population of 167 recombinant inbred lines (RILs), developed by ICARDA (Center for Agricultural Research in Dry Areas) and CIMMYT (International Maize and wheat Improvement Center), was used. This population was developed from a cross between 'Tadmor' and 'Er/Apm'. 'Tadmor' is adapted to the drought condition of the Middle-East (Grando, 1989).

Cloning and sequencing of RAPD bands

Genomic DNA was extracted from young leaves of each RILs and their parents using the CTAB methods described by Rogowsky et al. (1991). RAPD markers were generated for each DNA sample using 45 cycles of 1 min. at 94 °C, 1 min 32°C, 2 min at 72 °C followed by 1 cycle of 7 min at 72 °C. Fragment of size ~600 bp and ~800 bp generated by E9 and A19 RAPD markers, respectively, were excised from 1.0% agarose and then purified (Wizard® SV Gel and PCR Clean-Up System; Promega; Madison, USA). These two fragments are linked to drought tolerant related traits (RWC, OP and NL). Both fragment were then ligated into pGEM®-T Easy Vector (Promega; Madison, USA) and sequenced by-directionally at Macrogen Inc, Korea. Inserts were distinguished from vectors using VecScreen program in NCBI. For each cloned RAPD fragment, two pairs of 18-20 bp SCAR primers were designed with the first 10 bases matching the original 10 bases of the RAPD primer as possible. SCAR primer designing was performed using Primer3web (http://bioinfo.ut.ee/primer3) (Koressaar and Remm, 2007).

PCR mixed for SCAR markers contained 20 ng of plant genomic DNA, 5µl 5X buffer, 0.25µM MgCl2, 0.625 µM dNTP, 0.2 µM of primer and 1 unit of Taq DNA polymerase (Promega; Madison, USA). PCR protocol used an initial denaturation temperature of 95°C for 5 min followed by 35 cycles of 95°C for 30 s, 64°C for 1 min, and 72°C for 30 s with a final extension at 72°C for 4 min. in an Applied Biosystems 9700 PCR machine.

Map construction and QTL analysis

Amplified fragment length polymorphism (AFLP) analysis, map construction and QTL identification were performed as described by Akash, (2010) with modifications. In brief, linkage maps were constructed with Mapmaker3 software (Lander et al., 1987), using kosombi's map function (Kosombi, 1944) and minimum LOD score of 4. QTL analysis was performed with WinQTLCart software (Zeng, 1994; Zeng and Weir, 1996) using composite interval mapping.

3- Result and discussion

In the present study the conversion of two RAPD, E9 and A19 into SCAR markers was performed. Amplified products of size ~600 bp and ~800 bp generated by E9 and A19 RAPD markers linked to the drought tolerance traits (RWC, OP, NL) (Teulat et al., 1997; Teulat et al., 1998; Teulat et al., 2001) were cloned. Three transformed white colonies from each excised RAPD bands were selected for sequencing to insure that the correct fragment had been cloned and sequenced. The presence of white colonies indicated the insertion of foreign DNA and the loss of the cells ability to hydrolyse the X-gal. The X-gal was used to indicate whether a cell expressed the β-galactosidase enzyme, which was encoded by the *lacZ* gene, in a technique called blue/white screening. Two 18-20 bp SCAR primers were designed to contain the original 10 bp of the RAPD marker plus the internal 10-12 bp. To avoid possible secondary structure or primer dimer generation and false priming, Primer3web software was used to find the two SCAR primer combinations from each RAPD primer. One SCAR primer included the beginning of the sequence and the other SCAR marker included the end of the sequence (Table 1).

Table 1: Primer sequences for the SCARs derived from RAPD markers linked to drought tolerant traits in barley.

SCAR marker	Primer	Sequence (5' to 3')*	Expected size (melting temp.)	Linkage to drought tolerance traits
SCE9_600	SCE9_600F	TCACCCGAGCACTTGCAT	565(61)	OP & RWC
	SCE9_600R	CTTCACCCGACGACACTAGA		
SCA19_800	SCA19_800F	CAAACGTCGGCAATGGAG	777(61)	NL
	SCA19_800R	CAAACGTCGGGGTAGTAGAC		

*The sequences of the designed primers are listed with the RAPD primer sequence underlined. OP for osmotic potential at full turgor, RWC for relative water content, NL for number of leaves on the main stem.

The amplified fragment from E9 RAPD primer showed 95% homology with CBF12 (CBF12) gene in *Hordeum vulgare* subsp. vulgare retrotransposon associated with levels of freezing tolerance in temperate-climate cereals (Knox et al., 2010). However, no other known gene sequence in GenBank was found to show homology with sequence obtained from A19 RAPD primer. Possibly, it might be related to some conserved sequences of

barley genome, still unidentified for their genetic function.

Both generated SCAR bands were used as dominant maker and scored as present or absence for the RIL population. Since only a single fragment is amplified in dominant SCAR marker, post amplification electrophoresis can be eliminated as the PCR products can be stained and detected directly in a microtiter plate or by measuring DNA concentration with spectrophotometer (Weeden, 1994).

Iruela *et al.* (2006) reported that some SCAR makers were elongated not from base one from RAPD primers and in one of the SCAR markers they didn't use any base of RAPD primer to develop reverse SCAR primer. However, Scheef *et al.* (2003) reported two SCAR markers were all 10 bp RAPD primer elongated to develop to SCAR markers and the annealing temperature was close in reverse and forward primers in the same SCAR marker.

One of the difficulties of RAPD conversion to SCAR marker is that desirable cloning of the polymorphic bands cannot be frequently conducted due to the heterogeneous nature of polymorphic bands. In the procedure of converting randomly amplified polymorphisms to SCAR markers, non-targeted sequences can frequently be generated from heterogeneously amplified fragments of similar size with the specific fragment, which might be contained in the polymorphic product determined as one band on the gel images (Lee et al., 2010). In addition, loss of the initial polymorphism has been frequently reported (Paran and Michelmore 1993; Deng et al. 1997; Hernandez et al. 1999; Chowdhury et al. 2001). This lack of polymorphism may occur when the polymorphisms of RAPD primers are due to mismatches at the priming sites (Paran and Michelmore 1993).

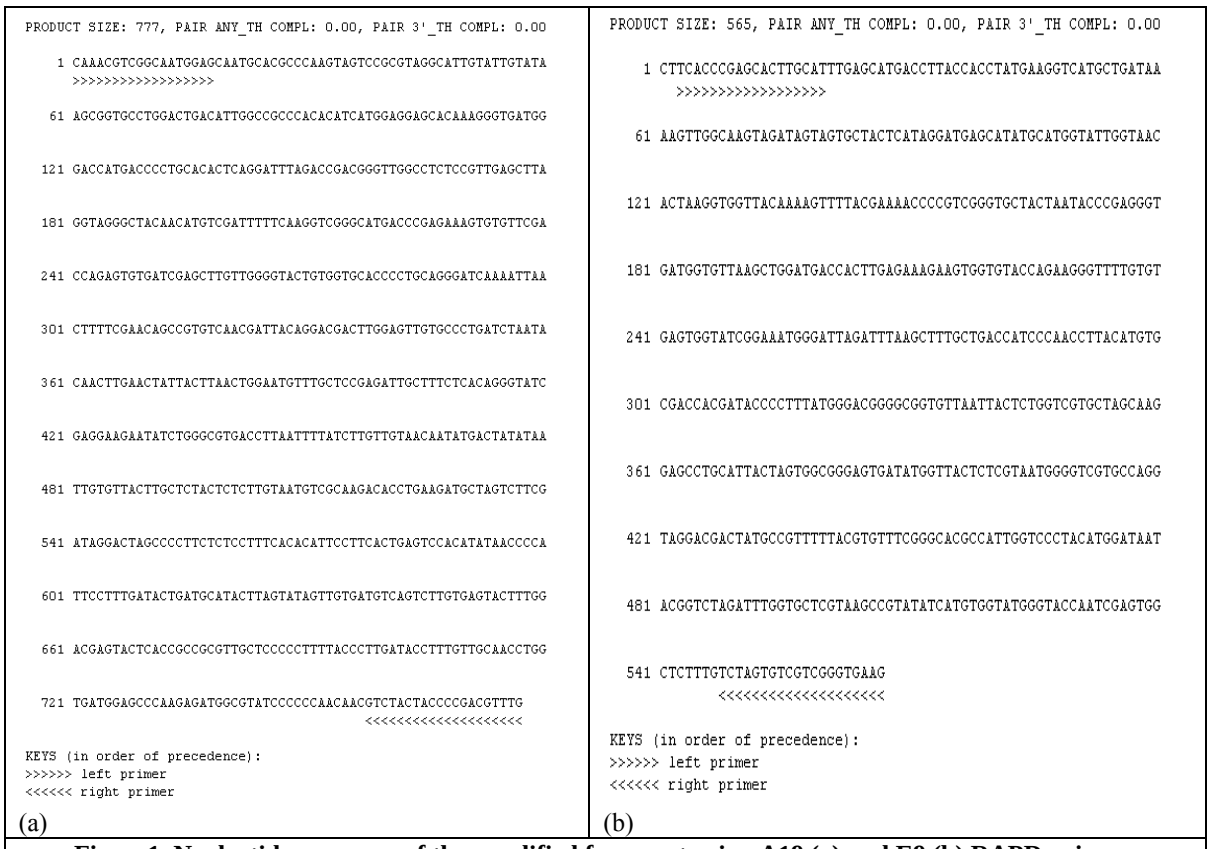

Figure1. Nucleotide sequence of the amplified fragment using A19 (a) and E9 (b) RAPD primers

Testing designed SCAR primers

SCA19_800 was mapped to linkage group number 1 (Figure 2). However, this mapped SCAR marker was not linked to kernel color studied by Akash (2010). SCAR markers were used in identifying many traits such as ploymembryony in citrus (Kang et al., 2008), male sterility in chili pepper (Lee et al (2010), resistance to Gall Midge in rice (Sardesai, et al., 2002) and Resistance to root knot nematode in pepper (Wang et al., 2009). In

barley, SCAR markers linked to scald resistance gene have been developed (Genger, et al., 2003). Also, four SCAR markers were used to construct a core genetic map of *Hordium chilense*. Also, genetic variation in powdery mildew was studied using SCAR, RAPD, and VNTR markers. In addition to their use as genetic markers, SCAR markers are useful in physical mapping. SCARs bridge the gap between the ability to obtain molecular markers linked to genes of interest in a short time and the use of these markers in a map-based cloning approach (Paran and Michelmore, 1993). This is the first attempt of SCAR markers development from RAPD markers linked to the drought tolerant traits (RWC, OP, NL). These reliable and reproducible markers can be used as selection tools to facilitate breeding programs for drought tolerance in barley.

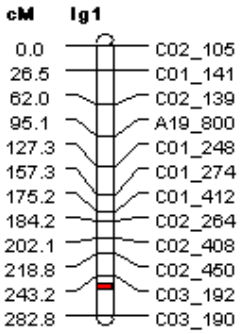

Figure 2. Linkage group of the barley RIL population derived from a cross between 'Tadmor' (drought tolerant) and 'Er/Apm' (drought susceptible) parents. Red bar indicates the position of a QTL detected by Akash, (2010).

References

References

Akash, M.W. 2010. Identifying QTL controlling kernel color in barley. J Crop Impr. 24: 219-227.

Ardiel, G.S., Grewal, T.S., Deberdt, P., Rossnagel, B.G., Scoles, G.J. 2002. Inheritance of resistance to covered smut in barley and development of a tightly linked SCAR marker. Theor. Applied Genet. 104: 457-464.

Blum, A. 1989. Osmotic adjustment and growth in barley genotypes under drought stress. Crop Sci. 29: 230-233.

Chowdhury, M.A., Andrahennnadi, C.P., Slinkard A.E., Vandenberg A. 2001. RAPD and SCAR markers for resistance to ascochyta blight in lentil. Euphytica 118: 331-337.

Deng, Z., Huang, S., Xiao, S., Gmitter, F.G, 1997. Development and characterization of SCAR markers linked to the citrus tristeza gene from Poncirus trifoliata. Genome 40: 697-704.

Eckstein, P.E., Krasichynska, N., Voth, D., Duncan, S., Rossnagel, B.G., Scoles, G.J. 2002. Development of PCR-based markers for a gene (*Un8*) conferring true loose smut (*Ustilago nuda* (Jens.) Rostr.) resistance in barley. Can J Plant Pathol. 24: 46-53.

Genger, R.K., Brown, A.H.D., Knogge, W., Nesbitt, K., Burdon, J.J. 2003. Development of SCAR markers linked to a scald resistance gene derived from wild barley. Euphytica 134:149-159.

Grando, S. 1989 Breeding for low rainfall areas. In: Cereal Improvement Program Annual Report. Aleppo, Syria, ICARDA 26-35.

Hernandez, P., Martin, A., Dorado, G. 1999. Development of SCARs by direct sequencing of RAPD products: a practical tool for the introgression and marker assisted selection of wheat. Mol. Breeding 5:245–253

Iruela, M., Rubio, J., Barro, F., Cubero, J.I., Millan, T., Gil, J. 2006. Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer arietinum L.*). Theor. Appl. Genet. 112; 278-287.

Kang, S.K., Yun, S.H., Lee, D.H. 2008. Development a SCAR marker linked to polyembryonic trait in cittus. Korean J Hortic. Sci. 26: 51-55.

Kasai, K., Morikawa, Y., Sorri, V.A., Valkonen, J.P.T. 2000. Development of SCAR markers to the PVY resistance gene Ryadg based on a common feature of plant disease resistance genes. Genome 43: 1-8.

Knox, A.K., Dhillon, T., Cheng, H., Tondelli, A., Pecchioni, N., Stockinger, E.J. 2010. CBF gene copy number variation at Frost Resistance-2 is associated with levels of freezing tolerance in temperate-climate cereals. Theor. Appl. Genet. 121: 21-35.

Koressaar, T., Remm, M. 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics 23:1289-1291.

Kosombi, D.D. 1944. The estimation of map distances from recombination values. Ann Eugen 12:172-177.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., Newburg, L. 1987. Mapmaker: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1: 174-181.

Lee, J., Yoon, J. B., Han, J.H., Lee, W.P. 2010. A codominant SCAR marker linked to the genic male sterility gene ms(1) in chili pepper (*Capsicum annuum*). Plant Breeding 129: 35-38.

Matin, M.A., Brown, J.H., Ferguson, H. 1989. Leaf water potential, relative water content, and diffusive resistance as screening techniques for drought resistance in barley. Agron. J. 81: 100-105.

Paran, I., Michelmore, R. 1993. Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor. Appl. Genet. 85: 985-993.

Rogowsky, P. M., Guidet, F.L.Y., Langridge, P., Shepherd, K.W., Koebner, R. M. D. 1991. Isolation and characterization of wheat-rye recombinants involving chromosome arm 1DS of wheat. Theor. Appl. Genet. 82: 537-544.

Sardesai, N., Kumar, A., Rajyashri, K.R., Nair, S. 2002. Identification and mapping of an AFLP marker linked to GM7, a gall midge resistance gene and its conversion to a SCAR marker for its utility in marker aided selection in rice. Theor. Appl. Genet. 105: 691-698.

Scheef, E.A., Casler, M.D., Jung, G. 2003. Development of Species-Specific SCAR Markers in Bentgrass. Crop Sci. 43: 345-349.

Teulat, B., Borries, C., This, D. 2001. New QTLs identified for plant water-status, water-soluble carbohydrate and osmotic adjustment in a barley population grown in a growth-chamber under two water regimes. Theor. Appl. Genet. 103: 161-170.

Teulat, B., This, D., Khairallah, M., Borries, C., Ragot, C., Sourdille, P., Leroy, P., Monneveux, P., Charrier, A. 1998. Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. 96: 688-698.

Teulat, B., Monneveux, P., Wery, J., Borries, C., Souyris, I., Charrier, A., This, D. 1997. Relationships between relative water content and growth parameters under water stress in barley: a QTL study. New Phytol. 137: 99-107.

Wang, L.H., Gu, X.H., Hua, M.Y., Mao, S.L. 2009. A SCAR marker linked to the N gene for resistance to root knot nematodes (Meloidogyne spp.) in pepper (Capsicum annuum L.). Sci. Hortic. Amsterdam 122: 318-322.

Weeden, N.F. 1994. Approaches to mapping in horticultural crops. In: Greshoff, P.M. (ed) Plant genome analysis. CRS Press, Boca Raton FL, pp 57-68.

Yasseen, B.T., Al-Maamari, B.K.S. 1995. Furthet evaluation of the resistance of black barley to water stress: Preliminary assessment for selecting drought resistant barley. J Agron. & Crop Sci. 174: 9-19 .

Zeng, Z.B. 1994. Precision mapping of quantitative trait loci. Genetics 140: 745-754.

Zeng, Z.B., Weir, B.S. 1996, Statistical methods for mapping quantitative trait loci. Acta Agron. Sin. 22: 535-549.

12/22/2013