Identification of RAPD and ISSR markers associated with flag leaf senescence under water-stressed conditions in wheat (*Triticum aestivum* L.)

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Abstract

Time of drought leaf senescence is an important determinant of yield under stress and optimal environments. A segregating population from a cross between drought sensitive genotype (Variant-1) and drought tolerant genotype (Veery), was made to identify molecular markers linked to flag leaf senescence gene in wheat under water-stressed conditions as indicator for drought tolerance. Thirty eight RAPD and twenty-five ISSR primers were tested for polymorphism among parental genotypes and F₂ population. The present study indicated that four RAPD and two ISSR markers linked to the flag leaf senescence gene in wheat. QTLs for flag leaf senescence were associated with RAPD markers (Pr11230bp, Pr19240bp, OPU06340bp and OPH13450bp) and explained 7.0%, 50.0%, 24.0% and 13.0% of the phenotypic variation, respectively. QTLs for flag leaf senescence were also associated with ISSR markers (M11100bp and AD2100bp) and explained 25% and 34% of the phenotypic variation, respectively. The RAPD markers (Pr11230bp, Pr19240bp, OPU06340bp and OPH13450bp) have genetic distance of 15.6, 15.0, 13.2 and 17.4 cm from flag leaf senescence gene, respectively. The ISSR markers (M11100bp and AD2100bp) also showed the genetic distance of 12.5 and 10.2 cm from flag leaf senescence gene, respectively. Therefore, these four RAPD and the two ISSR markers were linked to the QTL for the flag leaf senescence gene as indicator of drought tolerance. These markers can be used in wheat breeding programs, as a selection tool in early generations.

**Keywords:** flag leaf senescence; ISSR markers; RAPD markers; *Triticum aestivum* L.

**Abbreviations:** ISSR_inter simple sequence repeats; QTL_quantitative trait loci; RAPD_random amplified polymorphic DNA.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop in the world. Wheat production under limited rain or irrigation condition has become an objective in Egypt as well as many areas worldwide due to limitation of water supply. Wheat production is adversely affected by drought in 50% of the area under production in the developing and 70% in the developed countries (Trethowan and Pfeiffer, 2000). Genetic gain for yield in these environments is estimated about half of the yield compared to well-watered environments (Rajaram et al., 1996). It is difficult to make progress for grain yield and yield components under drought as they are complex characters influenced by many environmental factors and are characterized by low heritabilities and large genotype environment interactions under drought conditions (Smith et al., 1990). Recently, Tuberosa and Salvia (2007) reported that genomics based approaches provide access to agronomically desirable alleles present at quantitative trait loci (QTLs) that affect such responses, thus enabling us to improve the drought tolerance and yield of crops under water limited conditions more effectively. Molecular markers associated with quantitative trait loci (QTL) for drought adaptive traits could greatly enhance progress in breeding for drought tolerance. Molecular markers improve the efficiency of breeding by allowing manipulation of the genome through marker-assisted selection. In wheat (*Triticum aestivum* L.), flag leaf senescence (FLS) is related to the period of reallocating resources from the source to the sink during the grain filling. Since the flag leaf photosynthesis in wheat contributes about 30–50% of the assimilates for grain filling (Sylvester-Bradley et al., 1990), the onset and rate of senescence are important factors for determining yield potential (Evans, 1993). Though, quantitative trait loci mapping for FLS as a yield determinant in winter wheat under optimal and drought-stressed environments, have been studied using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers, which revealed the genetic control of this trait and the QTLs identified on chromosome 2D associated with better performance under drought (Verma et al., 2004). To date, there has been no report of QTL mapping for flag leaf senescence using RAPD and ISSR markers. The simplicity and applicability of the RAPD and ISSR techniques have been emphasized in this study. RAPDs, involving the use of single short DNA primer to direct amplification of discrete sequences, have shown promises in cereals (D’Ovidio et al., 1990; Devis et al., 1992). ISSRs have been used successfully in genome mapping for a variety of crop species including maize, rice, barley and wheat (Ben El Maati et al., 2004; Barakat et al.,...
heat flux density (MJ m⁻² h⁻¹), Thr = mean hourly air temperature (°C), $\Delta$ = Saturation slope vapor pressure curve at Thr, $\gamma$ = psychrometric constant (Kpa°C⁻¹ ), $\varepsilon$ (Thr) = saturation vapor pressure at air temperature Thr, ea = average hourly actual vapor pressure, u₂ = average hourly actual wind speed (ms⁻¹). Calculated ETo, (crop evapotranspiration), which is equal to 100% of daily water consumption for the wheat was used to calculate irrigation requirements with the following equation: Daily irrigation requirements (IR) = ETo + 15% (leaching requirements). The data of daily IR was adjusted to the volume of polyethylene bags used and the following (Table 1) show the volume of daily IR in cubic cm until the stage of flag leaf appearance then drought tests were carried out for 21 days. After 21 days from the stress condition, the flag leaf of the main tiller of each plant was selected during morning hours when leaves were fully turgid. The percentage of flag leaf area and remaining green (% GFLA) was measured using the leaf area meter (Portable Living Leaf Area Meter, Model: YMJ, Zhejiang Top Instrument Co., Ltd.). These assessments were carried out by the same operator in the population to avoid any bias between operators influencing results.

**DNA extraction**

Genomic DNA was extracted from fresh leaves of F₂ individual plants and their parents, using the Saghai-Maroof et al. (1984) CTAB method. RNA was removed from the DNA preparation by adding 10 µl of RNAase (10 mg/ml) and then incubated for 30 min at 37°C. DNA sample concentration was quantified by a spectrophotometer (Beckman Du-65). The reagents were obtained from Pharmacia Biotech (Amersham Pharmacia Biotech Ltd., UK).

**PCR amplification**

Thirty-eight RAPD and 25 ISSR primers (Barakat et al., 2010) were used in the present investigation to amplify the template DNA. The PCR reaction mixture consisted of 20-50 ng genomic DNA, 1×PCR buffer, 2.0 mM MgCl₂, 100 µM of each dNTP, 0.1 µM primer and 1U Taq polymerase in a 25 µL volume. Template DNA was initially denatured at 94°C for 4 min, followed by 45 cycles of 94°C for 1 min, 36°C (for RAPD analysis) or 50°C (for ISSR analysis) for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplification products were fractionated on 1% (for RAPD analysis) or 2% (for ISSR analysis) agarose gel.

**Bulked segregant analysis**

Bulked–segregant analysis (BSA) was used in conjunction with RAPD and ISSR analysis (Michelmore et al., 1991) to find markers linked to flag leaf senescence gene as indicator for drought tolerance. Tolerant and sensitive bulks were prepared from F₂ individuals by pooling aliquots, containing equivalent amounts of total DNA, approximately, 50 ng/µl from each of ten sensitive and ten tolerant F₂ plants selected, based on initial phenotypic assessments. RAPD and ISSR primers were, then, screened on the parents and the two bulk DNA samples, from which some primer combinations revealed bands that were polymorphic, not only among parental genotypes, but also between the pair of the bulk DNA. Based on the evaluations of DNA bulks, individual F₂ plants were analyzed with co-
Table 2. QTL analysis and significant association between drought tolerance and markers (RAPD and ISSR) in the 100 F2 plants population of Veery × Variant-1, using Chi – square ($\chi^2$), correlation (r) and coefficient of determination (R$^2$) analysis

<table>
<thead>
<tr>
<th>Markers</th>
<th>Tested of plant</th>
<th>Expected Ratio</th>
<th>$\chi^2$</th>
<th>r</th>
<th>R$^2$</th>
<th>QTL cM</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAPD</td>
<td>Pr11230bp</td>
<td>100 80 20</td>
<td>3 : 1</td>
<td>1.33$^{**}$</td>
<td>0.26</td>
<td>0.07$^{**}$</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>Pr19240bp</td>
<td>100 67 33</td>
<td>3 : 1</td>
<td>3.41$^{**}$</td>
<td>-0.71</td>
<td>0.50$^{**}$</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>OPU06340bp</td>
<td>100 67 33</td>
<td>3 : 1</td>
<td>3.41$^{**}$</td>
<td>0.49</td>
<td>0.24$^{**}$</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>OPH13450bp</td>
<td>100 64 36</td>
<td>3 : 1</td>
<td>6.4$^{**}$</td>
<td>-0.36</td>
<td>0.13$^{**}$</td>
<td>17.4</td>
</tr>
<tr>
<td>ISSR</td>
<td>M113100bp</td>
<td>100 67 33</td>
<td>3 : 1</td>
<td>3.41$^{**}$</td>
<td>0.50</td>
<td>0.25$^{**}$</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>AD2300bp</td>
<td>100 76 24</td>
<td>3 : 1</td>
<td>0.05$^{**}$</td>
<td>-0.58</td>
<td>0.34$^{**}$</td>
<td>10.2</td>
</tr>
</tbody>
</table>

T=Tolerant plants , S= sensitive plants; Ns , ** non significant and significant at .01 level of probability

Table 3. Analysis of variance of the difference for flag leaf senescence as determinant for drought tolerance in F2 population

<table>
<thead>
<tr>
<th>Marker</th>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
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<tbody>
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<td>520.75</td>
<td>520.75</td>
<td>4.578</td>
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<td></td>
<td>Error</td>
<td>98</td>
<td>11146.5</td>
<td>113.73</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>99</td>
<td>11667.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr19240bp</td>
<td>Genotypes</td>
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<td>4057.45</td>
<td>4057.45</td>
<td>52.25</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
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<td>7609.81</td>
<td>77.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>OPU06340bp</td>
<td>Genotypes</td>
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<td>3029.35</td>
<td>3029.35</td>
<td>34.37</td>
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</tr>
<tr>
<td></td>
<td>Error</td>
<td>98</td>
<td>8637.91</td>
<td>88.14</td>
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<td></td>
<td>Total</td>
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<tr>
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<td>3208.85</td>
<td>37.18</td>
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<tr>
<td></td>
<td>Error</td>
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<td></td>
<td>Total</td>
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<td></td>
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<td></td>
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<tr>
<td>M113100bp</td>
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<td>3089.19</td>
<td>35.29</td>
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<tr>
<td></td>
<td>Error</td>
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<td>8578.07</td>
<td>87.53</td>
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</tr>
<tr>
<td></td>
<td>Total</td>
<td>99</td>
<td>11667.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AD2300bp</td>
<td>Genotypes</td>
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<td>4073.52</td>
<td>4073.52</td>
<td>52.57</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Error</td>
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<td>7593.74</td>
<td>77.49</td>
<td></td>
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<tr>
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<td>Total</td>
<td>99</td>
<td>11667.25</td>
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</tr>
</tbody>
</table>

segregating primers (Barakat et al., 2010) to confirm linkage of RAPD and ISSR markers to the drought tolerant genes.

Data analysis

Goodness of fit to a 3:1 ratio was calculated for RAPD and ISSR markers by Chi-square test. The association between molecular markers (RAPD and ISSR ) and the flag leaf senescence as indicator for drought tolerance genes was assessed with correlation and simple regression analysis, using PROC REG of SAS version 9.1 software packages (SAS Institute, Cary, NC, 2007). Magnitude of the marker associated phenotypic effect was described by simple correlation (r) and the coefficient of determination (R$^2$), which represented the fraction of variance explained by the polymorphism of the marker. Single-marker analysis to detect main effect of QTL was performed by the method of Liu (1998). Significant association of a tested marker with a QTL for flag leaf senescence gene was detected by single-factor ANOVA.

Linkage analysis

Map manager QTX Version 0.22 (Meer et al., 2001) was used to analyze the linkage relationship of RAPD and ISSR markers detected from bulked segregate analysis. Linkage was detected when a log of the likelihood ratio (LOD) threshold of 3.0 and maximum distance was 50 cM. The Kosambi's mapping function was used. The chromosomal location of the QTLs for flag leaf senescence was further confirmed by the simple interval mapping method using QGENE program (Nelson, 1997).

Results

**RAPD markers analysis**

Out of 38 arbitrary primers screened for polymorphisms between (Veery and Variant-1 ), 24 RAPD primers (63.2 %), with higher polymorphic bands were suitable to differentiate between the two parents. Of these 24 RAPD primers, Pr11
primer (5’ CAATCGCCGT 3’), produced one strong polymorphic band at 230 bp, that was present only in the tolerant parent (Veery), as shown in Figure (1). The Pr11 primer was selected for screening DNA bulks and their parental DNA. The Pr11 primer, generated one polymorphic fragments at 230 bp, which was presented only in tolerant bulk and Veery (tolerant parent) whereas missing in sensitive bulk and Variant-1 (sensitive parent), as shown in Fig 1. In addition primer Pr19 (5’ CAAAGCCTCGG 3’), produced a strong polymorphic band at 240 bp that was present only in the sensitive bulked DNA, but not in the tolerant bulked DNA. Primer OPU06 (5’ ACCTTTGGCGG 3’), produced a strong polymorphic band at 340 bp that was present only in the sensitive bulked DNA, but not in the tolerant bulked DNA. Also, primer OPH13 (5’ GACGCCACAC 3’), produced a strong polymorphic band at 450 bp that was present only in the sensitive bulked DNA, but not in the tolerant bulked DNA. These RAPD markers (Pr11230bp, Pr19240bp, OPU06340bp and OPH13450bp) were regarded as candidate markers, linked to the flag leaf senescence gene as indicator for drought tolerance. These polymorphic markers; viz, Pr11230bp, Pr19300bp, OPU06340bp and OPH13450bp were further used to check their linkage to the flag leaf senescence gene, using a segregating F2 population, derived from the cross between the tolerant parent (Veery) and the sensitive parent (Variant-1). When analyzing the individual plants of F2 population, the Pr11230bp and OPU06340bp fragments were amplified and obtained only in F2 tolerant individuals. In addition Pr19300bp and OPH13450bp fragments were amplified in the DNA, obtained only in F2 sensitive plants. For the RAPD markers, Pr11230bp, Pr19300bp, OPU06340bp and OPH13450bp, 80, 33, 67 and 36 of 100 individuals exhibited the amplified polymorphic fragments, in the F2 population, respectively (230, 240, 340 and 450bp), while the remaining did not. The ratio fitted the expected Mendelian ratio (3:1) for all markers except for OPH13450bp which did not fit accordingly (Table 2). To check for potential co-segregation of DNA fragments and drought tolerant phenotypes, correlation and simple regression analysis were carried out in order to confirm association among the Pr11230bp, Pr19300bp, OPU06340bp and OPH13450bp markers and the flag leaf senescence gene as indicator for tolerant to drought in all 100 F2 progenies. The results showed that the correlation and the coefficient of determination (R²) for the relationship between the four markers (Pr11230bp, Pr19300bp, OPU06340bp and OPH13450bp) and the phenotypes of F2 individuals were significant and r = 0.26, -0.71, 0.49 and -0.36 were recorded, respectively. The coefficients of determination (R²) were also 0.07, 0.50, 0.24 and 0.13, for these markers, respectively (Table 2) which indicates that the four markers were associated with the flag leaf senescence gene as indicator for drought tolerance.

**ISSR marker analysis**

Out of 33 ISSR primers, screened for polymorphisms between the two tested parents, thirteen ISSR primers (34.2 %) with higher polymorphic bands were suitably recognized to differentiate between the two parents. Of these thirteen ISSR primers, M1 and AD2 (5’(AC) 8CG3’ and 5’(AGC) 6G3’, respectively), which produced two strong polymorphic bands at 1100 and 300 bp, respectively. The M1 primer produced one strong polymorphic band at 1100 bp, that was present only in the tolerant parent (Veery), as shown in Figure (1). The M1 primer was selected for screening DNA bulks and their parental DNA. The M1 primer, generated one polymorphic fragments at 1100 bp, which was presented only in tolerant bulk and Veery (tolerant parent) whereas missing in sensitive bulk and Variant-1 (sensitive parent), as shown in Fig 1. In addition primer M19 (5’ CAAAGCCTCGG 3’), produced a strong polymorphic band at 240 bp that was present only in the sensitive bulked DNA, but not in the tolerant bulked DNA. Primer OPU06 (5’ ACCTTTGGCGG 3’), produced a strong polymorphic band at 340 bp that was present only in the sensitive bulked DNA, but not in the tolerant bulked DNA. Also, primer OPH13 (5’ GACGCCACAC 3’), produced a strong polymorphic band at 450 bp that was present only in the sensitive bulked DNA, but not in the tolerant bulked DNA. These RAPD markers (Pr11230bp, Pr19300bp, OPU06340bp and OPH13450bp) were regarded as candidate markers, linked to the flag leaf senescence gene as indicator for drought tolerance. These polymorphic markers; viz, Pr11230bp, Pr19300bp, OPU06340bp and OPH13450bp were further used to check their linkage to the flag leaf senescence gene, using a segregating F2 population, derived from the cross between the tolerant parent, Veery, and the sensitive parent, Variant-1. AD2 ISSR primer was selected for screening DNA bulks and their parental DNA. The AD2 primer generated one polymorphic fragment at 300 bp that was present only in the sensitive parent (Variant-1). AD2 ISSR marker was selected for screening DNA bulks and their parental DNA. The M1 primer, generated one polymorphic fragments at 1100 bp, which was present only in tolerant bulk and Veery (tolerant parent) and were missing in sensitive bulk and Variant-1 (sensitive parent), as shown in Fig 2. In addition, AD2 primer was generated one polymorphic fragment at 300 bp that were present only in the sensitive parent (Variant-1). AD2 ISSR marker was selected for screening DNA bulks and their parental DNA. The M1 primer, generated one polymorphic fragments with 300 bp length, which were present only in the sensitive bulk and Variant-1 (sensitive parent) and missing in tolerant bulk and Veery (tolerant parent). These ISSR markers (M11100bp and AD2300bp) were regarded as candidate markers linked to the flag leaf senescence gene as indicator for drought tolerance in wheat.

These polymorphic markers, M11100bp and AD2300bp, were further used to check their linkage to the flag leaf senescence gene on the segregating F2 population, derived from the cross between the tolerant parent, Veery, and the sensitive, Variant-1. Analysis of F2 population showed that the M11100bp fragments were only amplified in tolerant and AD2300bp in sensitive individuals. In the F2 population, the ISSR markers, of M11100bp and AD2300bp, exhibited the amplified polymorphic fragments at 1100 and 300 bp in 76 and 24 of 100 individuals respectively, while, the remaining did not. The ratio fitted the expected Mendelian ratio (3:1) (χ² = 3.41 and 0.05, P < 0.1 respectively) (Table 2). To check for potential co-segregation of DNA fragments and drought tolerant phenotypes, correlation and simple regression analysis were carried out in order to confirm an association between the each M11100bp and AD2300bp markers and the flag leaf senescence gene as indicator for the tolerance to drought in all 100 F2 progenies.
The results showed that the correlation and the coefficient of determination ($R^2$) for the relationship between presence of the two markers, $M1_{1100bp}$ and $AD2_{300bp}$, and the phenotypes of $F_2$ individuals were significant and they recorded $r = 0.5$ and $-0.58$, respectively whereas, $R^2 = 0.25$ and $0.34$, respectively (Table 2). This indicates that these two markers were linked to the flag leaf senescence gene as well.

**QTL analysis**

The linkage relationship between the RAPD markers ($Pr11_{1230bp}$, $Pr19_{240bp}$, $OPU06_{340bp}$ and $OPH13_{450bp}$) and the flag leaf senescence gene as indicator for drought tolerance was estimated, using $F_2$ population, derived from the cross, Veery X Variant-1. The genetic distance between RAPD markers ($Pr11_{1230bp}$, $Pr19_{240bp}$, $OPU06_{340bp}$ and $OPH13_{450bp}$) and the flag leaf senescence gene were determined to be 15.6, 15.0, 13.2 and 17.4 cM with LOD scores of 16.4, 19.0, 20.7 and 16.0, respectively (Table 2 and Fig. 3). Therefore, RAPD markers ($Pr11_{1230bp}$, $Pr19_{240bp}$, $OPU06_{340bp}$ and $OPH13_{450bp}$ primers) were linked to the quantitative trait loci (QTL) for the flag leaf senescence gene as indicator for drought tolerance. After mapmaker linkage analysis on the $F_2$ population, the genetic distance between ISSR markers ($M1_{1100bp}$ and $AD2_{300bp}$) and the flag leaf senescence gene was determined to be 12.5 and 10.2 cM, respectively, with LOD scores of 21.6 and 26.0 respectively (Table 2 and Fig. 3). Therefore, ISSR markers ($M1_{1100bp}$ and $AD2_{300bp}$) were linked to the quantitative trait loci (QTL) for the flag leaf senescence gene as well. One-way ANOVA was carried out using marker genotypes as groups. The ANOVA on RAPD markers ($Pr11_{1230bp}$, $Pr19_{240bp}$, $OPU06_{340bp}$ and $OPH13_{450bp}$) and ISSR markers ($M1_{1100bp}$ and $AD2_{300bp}$) genotypes as groups for flag leaf senescence established significant association between marker (RAPD markers and ISSR markers) and phenotype (flag leaf senescence) (Tables 2 and 3). The single marker ANOVA analysis revealed that RAPD markers-linked QTL ($Pr11_{1230bp}$, $Pr19_{240bp}$ $OPU06_{340bp}$ and $OPH13_{450bp}$) accounted for 7.0%, 50.0%, 24.0% and 13.0% of the total variation of flag leaf senescence in $F_2$ population, respectively. While, ISSR markers-linked QTL ($M1_{1100bp}$ and $AD2_{300bp}$) accounted for about 25% and 34% of the phenotypic variation of leaf senescence in $F_2$ population, respectively, in wheat under water-stressed conditions.

**Discussion**

Water-stress tolerance in wheat is a quantitatively inherited trait controlled by several genetic loci which their genetic components are difficult to measure (Forster et al., 2000). Identification of associated molecular markers at a major locus contributing to water-stress tolerance would be useful for the indirect selection of wheat plants for water-stress tolerance (Visser, 1994). However, identifying molecular markers associated with important genes or traits in most instances requires screening of a relatively large number of individuals in the population (Lawson et al., 1994). Bulked segregant analysis (BSA) was originally developed to overcome this difficulty, because comparing bulk samples is easier than evaluating many individuals in different populations (Sweeney and Dannebeger, 1994). In this study, mapping quantitative trait loci for flag leaf senescence gene as indicator for drought tolerance in wheat under water-stressed conditions.

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**Fig 2.** ISSR fragments, produced by M1 (5’ (AC)8CG 3’), M: Molecular weight, followed by $P_1$ and $P_2$ parents Veery and Variant-1, respectively. Bt, bulk tolerant; Bs, bulk sensitive, F2 individuals in the cross (T: Tolerant; S: sensitive). Arrow points to polymorphic bands of the $M1_{1100bp}$ marker.

**Fig 3.** RAPD markers ($Pr11_{1230bp}$, $Pr19_{240bp}$, $OPU06_{340bp}$ and $OPH13_{450bp}$), ISSR markers ($M1_{1100bp}$ and $AD2_{300bp}$) and the flag leaf senescence gene were located through the MAPMAKER-QTL analysis. All distances are given in centi-Morgan, using Kosambi’s mapping function.
under drought stress are described in a segregating population (Veery × Variant-1). The plant population were genotyped by RAPD and ISSR markers. The timing of flag leaf senescence (FLS) is an important determinant of yield under stress and optimal environments. Cereal genotypes have been shown to exhibit differences in flag leaf senescence under drought, which affect yields such as sorghum (Rosenow and Clarke, 1981), maize (Baenziger et al., 1999) and durum wheat (Hafsi et al., 2000). QTL analysis based on a genetic map derived from 48 doubled haplaid lines using (SSR) markers, revealed the genetic control of this trait (Verma et al., 2004). The location of quantitative trait genes using QTL analysis is a labour and time consuming process since all the polymorphic markers are supposed to be screened on the segregating population. In recent years, some markers tightly linked to genes were found by using BSA (Xu et al., 1995; Mackay and Caligari, 2000; Zheng et al., 2002; Altinkue et al., 2003; Podlich et al., 2004; Govindaraj et al., 2005, Babiker et al., 2009). BSA was firstly reported by Michelmore et al. (1991) to identify RAPD markers tightly linked to genes for resistance to lettuce downy mildew. Using a method inspired by BSA, we are able to identify several types of molecular markers associated with flag leaf senescence in wheat under water-stressed conditions. These markers should be useful for marker-assisted selection. The present results support the idea that BSA can provide fast detection of molecular markers linked to genes of interest. Using BSA combining RAPD and ISSR markers analysis, we identified four RAPD markers (Pr11230bp Pr19240bp, OPU06340bp and OP1H13450bp) and two ISSR (ML1100bp and AD2300bp) linked to the flag leaf senescence gene as indicator for drought tolerance in wheat. In this study, QTLs for flag leaf senescence were associated with ISSR markers (M1 1100bp and AD2300bp) explained 25% and 34% of the phenotypic variation, respectively. While, QTLs for flag leaf senescence can be of great benefit to drought tolerance breeding programs as a selection tool in early generations.

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References


