P.24 벤타존 저항성 식물체 선발을 위한 AFLP 및 STS marker 개발

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Development of AFLP and STS markers specific to Bentazon in resistant wheat mutants

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1. Purpose

The purpose of this study was to identify herbicide resistant wheats among radiation mutated plants by using AFLP and its conversion of STS marker system. This sequence-specific PCR markers were used as an efficient tool in breeding programs for selection of herbicide Bentazon resistant wheat.

2. Materials and methods

1)Plant material : Geumgangmil(M_2 plant stage)

2)Mutagen source : The wheat samples were irradiated with the doses of 100, 150, 200, 250, 300, 400 Gy using the ^{60}Co source (γ-ray) at Korea Atomic Energy Research Institute.

3)Herbicide : Bentazon(3-isopropyl-2,1,3-benzothiadiazin-4-one-2,2-dioxide)

4)Selection : First selection - Evaluate germiability and early growth of M_2 plants treated with 7.5 times recommended concentration herbicide grown in cold room(4℃) supplied with B5 medium

Second selection - Scored activity of single leaf applied with 15 times recommended herbicide concentration for 36h in glass house.

5)AFLP analysis : Sixty four *EcoR* I /*Mse* I primer combinations were applied to develop AFLP markers specific to herbicide resistant wheat.

6)Conversion to STS marker : Targeted AFLP fragment were excised, ligated with pGEM-T Easy Vectors, cloned, sequenced, and sequence specific primers designed.

3. Results

◉ 575 plants among the 18,030 seeds were selected in first selection procedure and 4 plants were selected from 1st selected plants in 2nd selection procedure.

◉ Twenty nine primer combinations produced total twelve polymorphic products which were present in herbicide resistant mutation wheats but not in control plants(Fig 1). AFLP marker were used to design candidate STS primers of 18~25 bp in length. Only eight AFLP marker were suited for designing STS primers because they were over the 150bp(Table 1).

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◉ Especially, HRMW-08 primer set amplified unique polymorphic product present in herbicide resistant wheats but not in control plants(Fig 2). The PCR program was 4 min at 94℃ for pre-heating, followed 40 cycles of 30 sec at 94℃, 30 sec at 70℃, and 1 min at 72℃ for 4 min.
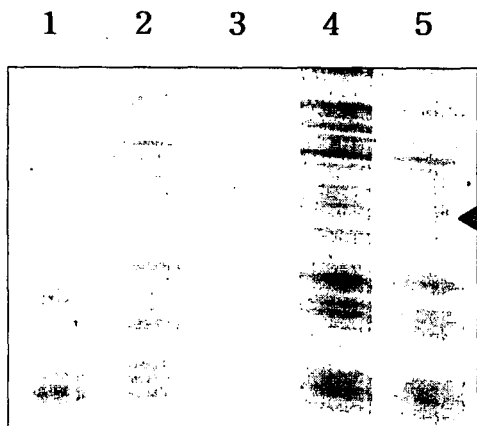

Fig 1. Variation in AFLP patterns between "Geumgangmil" and herbicide resistant wheat mutants. The arrow indicated an AFLP product unique to herbicide resistant wheat mutants. A band which was present in lane 2~5, but absent in lane 1.
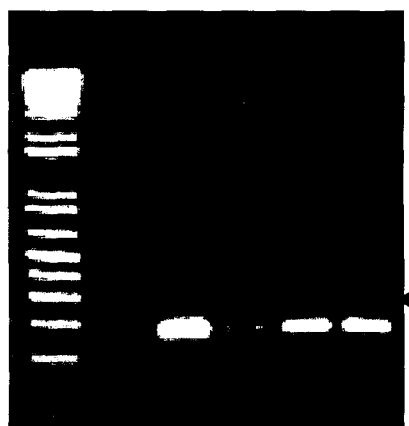Lane 1; control(Geumgangmil), lane 2~5; herbicide resistant mutation wheats.

Fig 2. PCR amplification products using primer(HRMW-08). The arrow indicated herbicide resistant mutation wheats specific band(175bp).
Lane 1; control(Geumgangmil), lane 2~5; herbicide resistant mutation wheats, M; molecular marker.

Table 1. Sequences of STS primers derived from AFLP polymorphic DNA fragments

AFLP primer	Name	Sequence of STS primer	Anneal. Temp.(℃)
E+AAC/M+CTG	HRMW-01	5′-GCGCGCACTGCACCACCCTTCG-3′ 5′-GCACCTTCAGCTGTAGAATCTA-3′	60
E+AAC/M+CTG	HRMW-02	5′-AGCCCGCGTGACGGTAACCGTT-3′ 5′-TTTTACATCCGACGGTCGGAG-3′	63
E+ACC/M+CAA	HRMW-03	5′-CACCCACTTTCAGTCAAT-3′ 5′-GACCACTGGTTTCAAGAAGGGC-3′	60
E+AAC/M+CTG	HRMW-04	5′-CGATGCCTGATCAGGTTTGATG-3′ 5′-GCCCTGGATCTGGAGCAGATT-3′	62
E+ACC/M+CAT	HRMW-05	5′-CAGACTTACAGAGGAATCGGC-3′ 5′-GGAGTGGAGTGGATATGTTACC-3′	64
E+ACC/M+CAG	HRMW-06	5′-ATGGGGGAACTACCTAACCT-3′ 5′-CCAAAGCTCCTTTTACCCAAGG-3′	52
E+ACT/M+CTT	HRMW-07	5′-GAGCTCCACAGTAGTTAGTAC-3′ 5′-GCATCACAGGTCCAGTGTTCAT-3′	54
E+ACC/M+CAA	HRMW-08	5′-GAGAAGATAAGAGATAACCGGCCC-3′ 5′-AGGTCTTGCTCAGCGTTGTCCACA-3′	70