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Anther culture properties of oat x wild red oat progenies and a search for RAPD markers associated with anther culture ability

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A study was carried out to improve anther culture ability of the non-responsive cultivated oat, *Avena sativa* L. cv. Puhti by introgressing favourable alleles from the responsive wild red oat, *Avena sterilis* L. acc. CAV 2648. Anther culture ability of these parental lines and F_2 progenies of their cross and two backcrosses was tested. Genotype effects were significant on all anther culture traits measured. The number of anther culture derived embryo-like structures was highest in acc. CAV 2648, and the number of green regenerants from the Puhti × CAV 2648 progeny. Anther culture response was greatly reduced in backcross progeny and was least in cv. Puhti. Random amplified polymorphic DNA (RAPD) was used to test for marker associations with oat anther culture traits in a population of 38 F_2 progenies. Two RAPD markers were putatively associated with improved production of green regenerants (one derived from acc. CAV 2648 and the other from cv. Puhti). One marker putatively associated with decreased albino plant regeneration (derived from acc. CAV 2648). These markers might be useful for selecting alleles for better anther culture ability among progeny of planned crosses. In addition, three markers, derived from acc. CAV 2648, were putatively associated with decreased anther culture response rates.

Key words: androgenesis, random amplified polymorphic DNA, genetic effects, genotypes, *Avena sativa, Avena sterilis*

Introduction

Doubled haploid (DH) plants represent useful material for genetical research and cultivar breeding programmes due to the complete homozygosity reached in a single generation. Anther culture, where plants are derived from haploid microspores, is a frequently used method to produce DH-plants (Sopory and Munshi 1996). It has been successful in oats (*Avena sativa* L.), but in recalcitrant genotypes green plant

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regeneration often occurs at low rates or not at all (Rines 1983, Sun et al. 1991, Kiviharju et al. 2000).

Genetic factors are known to influence anther culture ability in cereals and the choice of genotype is important when using this technique (e.g. Holme et al. 1999). For example, in anther culture of wheat (*Triticum aestivum* L.), genotype differences were reported to account for 32–85.6%, and in barley (*Hordeum vulgare* L.) 73%, of the total variation in green plant regeneration (Zhou 1996, Torp et al. 2001). Also in oats, evidence of the strong effect of genotype on anther culture response was reported (Rines 1983, Kiviharju et al. 1998). The efficiency of anther culture depends on induction of embryo-like structures, regeneration of plants from these structures and the ratio of green to albino plants regenerated. Reciprocal crosses in cereals have provided evidence that independently inherited, mainly nuclear, genes control these traits and there are both additive and non-additive genetic effects (Charmet and Bernard 1984, Tuvesson et al. 1989, Quimio and Zapata 1990, Zhou 1996, Torp et al. 2001). Chromosomes or chromosome regions influencing green plant regeneration in cereal anther culture have previously been located using translocation and substitution lines (De Buyser et al. 1992, Ghaemi et al. 1995). Progress has been made recently by applying DNA-based genetic marker technology (He et al. 1998, Yamagishi et al. 1998, Manninen 2000, Torp et al. 2001, Kwon et al. 2002).

One possible strategy to improve anther culture response is to introgress desirable alleles by crossing from genotypes known to respond well in anther culture. This method has been used successfully to improve anther culture ability of recalcitrant genotypes in other cereals eg. wheat (Hu 1997), barley (Ouédraogo et al. 1998) and rice (*Oryza sativa* L.) (He et al. 1998). In maize (*Zea mays* L.), anther culture results are improved by producing DH plants from different germplasms and intercrossing them (Marhic et al. 1998). Anther culture ability has been sought from more distant genepools: enhancement was obtained in recalcitrant *indica* rice by crossing with responsive *japonica* rice (Omar Faruque et al. 1998), and in rye (*Secale cereale* L.), by introgressing sections of the wheat genome through creating rye-wheat addition lines (Martinez et al. 1994). In oats, crossing the non-responsive cultivar Puhti with the responsive hexaploid wild red oat (*A. sterilis* L.) accession CAV 2648 resulted in regeneration of green plants following anther culture of the progeny (Kiviharju and Tauriainen 1999).

Marker assisted selection enables efficient breeding for targeted traits (Kwon et al. 2002). DNA markers associated with good anther culture ability can be selected for crossing parents and breeding lines. This approach would also support transfer of regeneration ability from responsive genotypes to recalcitrant ones through crossing (Beaumont et al. 1995, He et al. 1998, Yamagishi et al. 1998, Holme et al. 1999). In order to use this approach, genetic markers associated with anther culture traits need to be mapped. DNA markers and quantitative trait loci (QTLs) associated with anther culture ability traits have been reported in wheat, barley, maize and rice (Beaumont et al. 1995, Murigneux et al. 1994, He et al. 1998, Yamagishi et al. 1998, Manninen 2000, Torp et al. 2001, Kwon et al. 2002). In hexaploid oats, DNA markers associated with agronomically important traits, such as disease resistance (Penner et al. 1993, O'Donoughue et al. 1996, Bush and Wise 1998, Jin et al. 1998, Zhu et al. 2003, Zhu and Kaeppler 2003), quality (Holland et al. 1997, Kianian et al. 2000, Groh et al. 2001) and dwarfness (Milach et al. 1997) have already been reported. However, markers associated with anther culture traits of oats have not yet been published.

In this study, anther culture ability of oat cv. Puhti and wild red oat acc. CAV 2648, and their F_2 progeny and two backcross progeny populations was determined, in order to test if acc. CAV 2648 could be used as a donor of good anther culture ability to a recalcitrant genotype of cultivated oat. Some putative random amplified polymorphic DNA (RAPD) markers associated with anther culture ability traits of oats were identified based on F_2 progeny analysis.

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Material and methods

Oat cv. Puhti, red wild oat acc. CAV 2648, their cross and two backcross progeny populations were used as plant material in this study. F_2 generations were used, since only four F_1 plants were obtained despite numerous hybridisations made. Development of F_2, BC_1F_2 and BC_2F_2 generations is shown in Figure 1. Anther-donor plants were grown in a greenhouse under controlled conditions: 17/13˚C day/night temperatures, 16 hours photoperiod. The light intensity provided by fluorescent lamps was about 350 µmol m^{-2} s^{-1}. Seeds were sown in a peat soil mix (one seed in 14 cm pot) and fertilized with Nursery Stock Superex (N 19%, P 4%, K 20%, Kekkilä, Finland).

Anther culture was carried out according to Kiviharju et al. (2000). Tillers were cut and cold pretreated at +4˚C for 7 days. Anthers were isolated onto culture medium and heat pretreated at +32˚C for 5 days before culturing at +25˚C in the dark. Embryo-like structures were induced on double layer medium containing W_{14} salts and vitamins (Ouyang et al. 1989) supplemented with 5.0 mg l^{-1} 2,4-D, 0.5 mg l^{-1} kinetin and 10% maltose, and regenerated on solid medium containing 2.0 mg l^{-1} α-naphthaleneacetic acid (NAA), 0.5 mg l^{-1} kinetin and 2% sucrose (Kiviharju et al. 2000). To measure the anther culture properties of the cross parents, cv. Puhti and acc. CAV 2648, 1140 anthers (38 Falcon® Petri dishes, 3.5 cm in diameter, 30 anthers per dish) of each parent were isolated using numerous donor plants.

For the F_2, BC_1F_2 and BC_2F_2 populations, 38 plants were selected from each of the crosses as anther-donor plants, and their anther culture properties were determined by isolating and culturing 90 anthers (3 Petri dishes) of each of the selected plants. In total, the number of the isolated anthers was 3360 from the Puhti × CAV 2648 F_2 progeny, 3060 from the BC_1F_2 progeny and 3420 from the BC_2F_2 progeny. Anther culture traits recorded were number of embryo-like structures induced (ELS), number of embryo-like structures which reached the required size for transfer to the regeneration medium during eight weeks of culture (tELS), production of green (Green) and albino (Alb) regenerants and ploidy levels of the regenerated plants. The number of ELS (tELS added with the number of remaining ELS developed in 8 weeks) gives only indicative results, since numbers of the remaining ELS in some Petri dishes had to be estimated due to their high number.

Leaf material for DNA extractions was collected from plants of parents cv. Puhti and acc. CAV 2648, from the 38 F_2 plants used as anther-donors and from the DH regenerants derived from anther culture of these plants (Fig. 1.). The extracted DNA was stored at –70˚C. A rapid, small scale DNA extraction method of Edwards et al. (1991) was used. Homogenization of the leaf material was done using a Fastprep® homogenisator (Qbiogene, Inc). Due to the problems of DNA degradation in wild red oat, the DNA extraction method was replaced by the CTAB-method of Tinker et al. (1993), supplemented with RNAase A treatment (0.1 mg ml^{-1}). The DNA pellet was air dried and dissolved in 50 µL TE buffer. Equal amounts of DNA from nine CAV 2648 individuals were pooled for better coverage of the possible genetic variation still present in the wild red oat accession. Otherwise DNA samples from single plants were used.

RAPD analysis was based on methods reported by Fennimore et al. (1999), Penner et al. (1993) and Williams et al. (1991). Polymerase chain reaction (PCR) conditions were optimised for repeatable amplification of oat and wild red oat DNA. The volume of the PCR reaction mixture was 25µl, containing 0.8U *Taq*-polymerase purchased from MBI Fermentas, Tris-HCl -buffer containing $(NH_4)_2SO_4$ provided by the enzyme manufacturer, 2 mM $MgCl_2$, 100µM of each dNTP, 600–1200 nM of each primer and approximately 20–100 ng of sample DNA. The PCR program in the PTC-100™ Programmable Thermal Controller (MJ Research, Inc) consisted of 45 cycles of: 1 min at 94˚C for denaturation, 1 min 20 sec at 38˚C for annealing and 2 min at 72˚C for extension. The final extension step was 10 min at 72˚C. Annealing temperature was optimised by gradient-PCR (temperatures 32, 34,

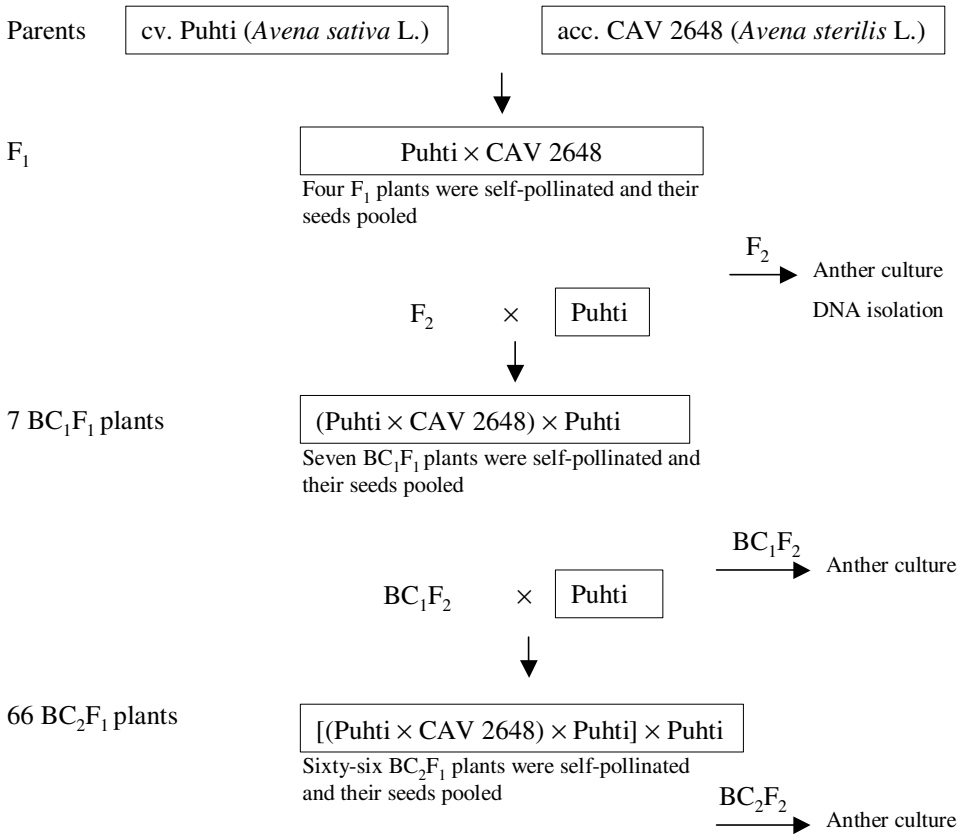

Fig. 1. Oat (*Avena sativa* L.) and wild red oat (*A. sterilis* L.) cross material used in this study was made according to this scheme.

36, 38 and 40˚C were tested). Raising the annealing temperature above 37˚C, as used by Fennimore et al. (1999), produced sharper bands and improved PCR reaction results, especially from wild red oat DNA.

Amplification products were separated by electrophoresis on 1.4% agarose gels (Seakem® LE agarose) with Tris-borate EDTA (TBE) buffer overnight (17h) at 50 V (constant voltage) or for 5h at 100 V. Ethidium bromide stained gels were visualized under UV-light using Eagle Eye™ (Stratagene).

One hundred and eighty six RAPD primers from Operon Technologies were tested to find markers that differed between the parents. The RAPD data were generated by scoring the absence of a DNA fragment as 0 and the presence of a band as 1. The best markers were selected for further studies. Plants producing unclear amplification products were re-analysed, and the promising marker $OPX\text{-}11_{870}$ (OPX-11 primer, 870 base pairs) was analysed twice. The 38 Puhti × CAV 2648 F_2 plants used as anther donors, were tested with the polymorphic markers. In order to find markers associated with good anther culture response traits, data produced from selected RAPD markers were compared statistically with the anther culture result data from each of these plants (means from three Petri dishes per anther-donor plant).

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Due to the non-normal distributions and non-equality of the variances in data, statistical significancies, based on χ^2-values, were determined using a generalized linear model, which was fitted by maximum likelihood estimation, applying a negative binomial distribution and logarithmic link function (PROC GENMOD, SAS Institute Inc., Cary, NC). Also a non-parametric Kruskal-Wallis test based on χ^2-test of rank sums (Wilcoxon scores) was applied (PROC NPAR1WAY, SAS Institute Inc., Cary, NC). A significance threshold of $P \leq 0.05$ was used. Linkages of significant RAPD markers were analysed using Mapmaker/Exp 3.0 and a LOD (logarithm of odds) score of 3.0 was used to establish linkage (Lander et al. 1987).

Results and discussion

Genotype had a statistically significant effect on all traits measured from anther culture of oat cv. Puhti, wild red oat acc. CAV 2648 and their cross and backcross progenies (Table 1), as expected from previous reports in other cereals (Zhou 1996, Torp et al. 2001). The number of ELS was significantly higher in anther culture of the responsive parent, acc. CAV 2648, compared with all other genotypes (Table 2). Although the number of ELS transferred on to the regeneration medium did not differ statistically between acc. CAV 2648 and the Puhti × CAV 2648 F_2 cross progeny, green plant regeneration rate was significantly better from anther culture of the F_2 cross progeny, compared with results from acc. CAV 2648 (Table 2). This is most probably explained by the beneficial effect of heterozygosity of the cross, as reported in barley, for example (Ouédraogo et al. 1998). However, in wheat cross progenies, heterosis was evident in formation of embryo-like structures, green plant regeneration rate being intermediate between that of the parents (Tuvesson et al. 1989, Holme et al. 1999).

Conversion rate of ELS to green plants was statistically higher for acc. CAV 2648 and F_2 and BC_1F_2 progenies of the parental genotypes compared with BC_2F_2 and cv. Puhti. The highest number of albino plants, as for the highest number of green regenerants, was recorded for F_2 plants, but albino numbers were generally low in the genotypes used. Progenies of the BC_1F_2 and BC_2F_2 produced significantly lower ELS, tELS and production rates of regenerated plants than the F_2 progeny of the parental genotypes. Cultivar Puhti, the non-responsive parent, gave statistically the worst results for all traits (except regeneration of green plants where the difference between its value of zero and the low

Table 1. Effect of the genotype of *Avena sativa* cv. Puhti, *A. sterilis* acc. CAV 2648 and their cross and backcross progenies on different anther culture response traits.

Characteristics	DF	χ^2	$P > \chi^2$
Embryo-like structures (ELS)	4	138.32	< 0.0001
Transferred embryo-like structures (tELS)	4	139.14	< 0.0001
Regenerated green plants (Green)	4	83.43	< 0.0001
Regenerated albino plants (Alb)	4	33.57	< 0.0001
Total number of plants regenerated (Tot)	4	89.35	< 0.0001
Percentage of green plants regenerated per transferred embryo-like structures (Green/tELS)	4	28.44	< 0.0001
Percentage of total number of plants regenerated per transferred embryo-like structures (Tot/tELS)	4	28.89	< 0.0001

Statistical analysis was done using a generalized linear model. Statistical differences between pairs are presented in Table 3. DF = degrees of freedom, χ^2 = Chi-Square test value.

Table 2. Results of anther culture of *A. sativa* L. cv. Puhti, *A. sterilis* L. acc. CAV 2648 and F_2 generations of their cross and two backcross progenies.

Genotype	Anthers	ELS		tELS		Green		Green final		Ploidy level			Green/ tELS		Albino	
	n	n	/100 anthers ± SE	n	/100 anthers ± SE	n	/100 anthers ± SE	n	/100 anthers ± SE	H n	DH n	DH %	%	n	/100 anthers ± SE
CAV 2648	1140	3613	316.9*a* ± 33.9	628	55.1*a* ± 6.6	14	1.2*a* ± 0.4	13	1.1*a* ± 0.4	13	0	0	2.2*a*	4	0.4*a* ± 0.2
F_2	3360	5734	170.7*b* ± 22.6	2344	69.8*a* ± 11.2	110	3.3*b* ± 0.6	104	3.1*b* ± 0.6	73	31*	30	4.7*a*	20	0.6 *a*± 0.2
BC_1F_2	3360	2161	64.3*c* ± 10.7	537	16.0*b* ± 3.3	16	0.5*c* ± 0.2	14	0.4*c* ± 0.2	11	3	21	3.0*a*	0	0*ab*
BC_2F_2	3420	1599	46.8*c* ± 10.7	254	7.4*c* ± 1.6	2	0.1*d* ± 0.0	2	0.1*d* ± 0.0	2	0	0	0.8*b*	2	0.1*b* ± 0.0
Puhti	1140	10	0.9*d* ± 0.3	4	0.4*d* ± 0.2	0	0*d*	0	0*d*	0	0	0	0*b*	0	0*b*

Statistical analysis was performed using a generalized linear model, and statistical differences among genotypes at $P < 0.05$ are indicated by different lower case letters. ELS = Embryo-like structures, tELS = transferred embryo-like structures, Green = regenerated green plants, Albino = regenerated albino plants, Green final = Greens minus plants dead during rooting or transferring in to the greenhouse. SE = standard error of the mean, H = haploid, DH = doubled haploid. * one additional unclear ploidy was recorded

value of the BC_2F_2 progeny was not significant). In general, anther culture response was reduced as the contribution of acc. CAV 2648 genome declined.

Based on our results, it is clear that if less responsive cv. Puhti is crossed with responsive acc. CAV 2648, an increase in frequency of particular alleles will increase the response level of the cross progeny dramatically, compared with cv. Puhti. This is in line with observations on anther culture of other cereals (Hu 1997, He et al. 1998, Ouédraogo et al. 1998). However, the rapid reduction in anther culture ability in backcross progenies would be problematic in attempts to transfer anther culture capacity to cultivated oat from wild red oat. Several backcrosses to cultivated oats are needed to remove unwanted genes of the wild red oat. However, markers associated with anther culture ability could help in selecting those progeny lines with alleles favouring anther culture ability. Variation between the parental genotypes was expressed in their cross progeny, and results for all anther culture traits, measured from the 38 Puhti × CAV 2648 F_2 anther-donor plants, differed significantly among these 38 genotypes (Table 3). Thus they could be used as material for finding markers associated with anther culture traits. Distribution of F_2 plant values for key components is shown in Figure 2 by box plots.

Screening 186 RAPD primers in cv. Puhti and acc. CAV 2648 resulted in seventeen primers (OPA-12, OPB-11, OPB-12, OPB-15, OPB-19, OPC-10, OPC-14, OPC-16, OPD-20, OPF-09, OPV-15, OPW-20, OPX-11, OPY-01, OPY-03, OPY-15, OPZ-20), which clearly differentiated the parental genotypes. Presence or absence of 53 markers, produced by these primers, was scored for all 38 F_2 anther-donor plants of the Puhti × CAV 2648 cross. When the results of these markers were compared with the anther culture data of the same plants, five markers, produced by four primers, were significantly ($P \leq 0.05$) associated with anther culture response traits (Table 4). Marker $OPX\text{-}11_{870}$, derived from acc. CAV 2648, was associated with better green and albino plant regeneration

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(Fig. 3). These traits were previously reported to be affected by the same QTL in rice (He et al. 1998). Surprisingly, marker OPC-10_{480}, associated with regeneration of green plants, sum of green and albino plants, as well as conversion rate of ELS to greens or to sum of green and albino plants, was derived from the cv. Puhti. The probable explanation for this is that due to inhibition of induction of ELS in anther culture of cv. Puhti, the effect of alleles favouring green plant regeneration could not be detected in this cultivar. Alleles favouring anther culture traits have been reported to derive from the less responsive parent also in other cereals including wheat (Torp et al. 2001), maize (Beckert 1998) and rye (Martinez et al. 1994). Marker OPC-14_{630}, from acc. CAV 2648, was associated with low albino plant production and could be useful when alleles causing high rates of albinism are selected out of the cross progeny. However, associations between markers and albinism were based only on a very low number of albino plants regenerated, which may affect their reliability. In addition to having favourable alleles, the responsive parent seemed to distribute alleles affecting anther culture negatively, reflecting complicated genetic regulation of anther culture traits. Two markers derived from the responsive wild red oat parent, OPY-03_{890} and OPC-14_{520}, were associated with inferior anther culture results: both with decreased induction of ELS, and the second of them also with lower green and albino plant regeneration rates, compared with the allele derived from cv. Puhti. According to our results, the optimal anther culture response, exceeding that of the responsive parent, could be achieved by combining the positive alleles from both cv. Puhti and acc. CAV 2648. However, further studies are needed to verify the allelic effects and to reveal the possible interactions between loci.

None of the five markers associated with anther culture traits were linked to each other. This may be because of the low sample number, only 38 plants were tested. On the other hand, in the case of dominant markers like RAPDs, the re-

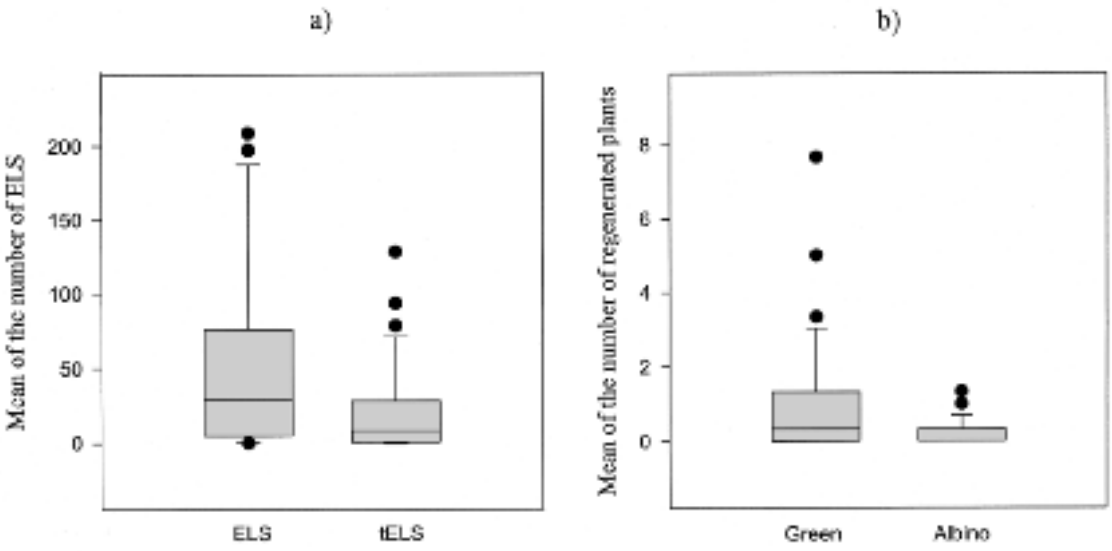

Fig. 2. Distributions of Puhti × CAV 2648 F_2 plant values for key response traits are presented by box plots for total number of developed embryo-like structures (ELS) and number of embryo-like structures transferred onto regeneration medium (tELS) in diagram a), and for number of regeneratad green (Green) and albino (Alb) plants from the transferred embryo-like structures in diagram b).

Table 3. Effect of the genotype of 38 Puhti × CAV 2648 F_2 plants on different anther culture response traits.

Characteristics	DF	χ^2	$P > \chi^2$
Embryo-like structures (ELS)	37	93.24	< 0.0001
Transferred embryo-like structures (tELS)	37	96.46	< 0.0001
Regenerated green plants (Green)	37	86.36	< 0.0001
Regenerated albino plants (Alb)	37	53.69	< 0.0374
Total number of plants regenerated (Tot)	37	83.14	< 0.0001
Percentage of green plants regenerated per transferred embryo-like structures (Green/tELS)	37	77.19	0.0001
Percentage of total number of plants regenerated per transferred embryo-like structures (Tot/tELS)	37	74.64	0.0004

Statistical analysis was done using a Kruskal-Wallis test. DF = degrees of freedom, χ^2 = Chi-Square test value.

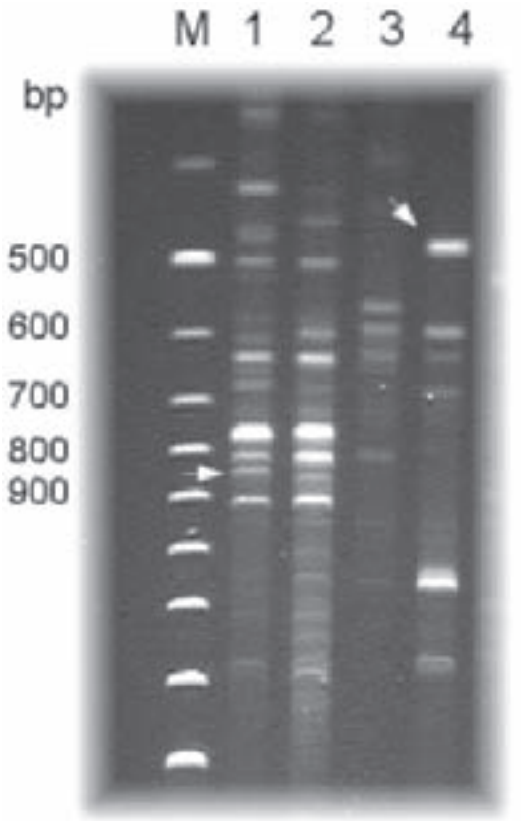

Fig. 3. Random amplified polymorphic DNA polymorphism between oat, *Avena sativa* L. parent cv. Puhti (lines marked by P) and wild red oat, *A. sterilis* L. parent acc. CAV 2648 (lines marked by C) with primers X11 and C10. Arrows indicate the marker bands associated with anther culture traits. Line M is the molecular weight marker GeneRuler™ 100 bp DNA Ladder Plus (MBI Fermentas).

pulsion phase (alleles derived from different parents) affects the LOD score due to inefficient estimation of recombination frequencies (Ott 1985). The segregation of four of the five markers (OPX-11_{870}, OPC-14_{520}, OPC-14_{630} and OPY-03_{890}) associated with anther culture traits did not fit the expected segregation ratio of alleles in the F_2 progeny (3:1 ratio, χ^2-test, $P \leq 0.05$), which is very common in anther culture derived DH-progenies (Manninen 2000). This segregation distortion could also be coincidental or caused by the use of an unknown number of acc. CAV 2648 plants as pollinators that were not pure lines. However, genes affecting different anther culture traits are not necessarily located close to each other in the genome. According to QTL mapping results, more than twelve loci on 9 chromosomes of maize (Beckert 1998, Marhic et al. 1998) and eight loci on seven chromosomes of rice (He et al. 1998) are estimated to control the androgenic pathway.

One CAV 2648 marker (OPX-11_{870}), associated with better production of green regenerants, was selected for closer examination and its reproducibility was determined. DH regenerants (n = 103) derived from anther culture of F_2 progeny of Puhti × CAV 2648 were tested with the primer OPX-11 to establish if this marker was more common in the regenerants. More than half of the DH-regenerants had the fragment: 62% (64 of 103 DH-plants) expressed the marker, whereas 38% did not. Unfortunately, possible

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Table 4. Putative RAPD markers associated with anther culture response traits of *Avena sativa* L. cv. Puhti × *A. sterilis* L. acc. CAV 2648 F_2 progeny plants.

Marker	Anther culture response	Response in plants having allele from CAV 2648		Response in plants having allele from Puhti		Statistically significant differences in response between the two alleles		Parent with the amplified marker band
		n	Mean	n	Mean	χ^2	$P > \chi^2$	
OPX-11_{870}	Green	21	4.71	17	1.37	5.10	0.0239	CAV 2648
	Alb	21	0.90	17	0.20	4.33	0.0374	
OPC-10_{480}	Green	10	0.89	28	4.05	5.27	0.0216	Puhti
	Green/tELS	10	1.24	28	5.87	5.33	0.0209	
	Tot/tELS	10	1.56	28	6.55	5.52	0.0188	
	Tot	10	1.11	28	4.76	5.61	0.0178	
OPC-14_{630}	Alb	20	0.28	16	1.04	4.21	0.0403	CAV 2648
OPY-03_{890}	tELS	10	28.78	26	89.10	4.45	0.0350	CAV 2648
OPC-14_{520}	tELS	20	36.56	16	117.01	5.83	0.0158	CAV 2648
	Green	20	1.67	16	5.56	5.39	0.0202	
	Alb	20	0.17	16	1.18	8.31	0.0039	
	Tot	20	1.83	16	6.74	7.55	0.0060	

Statistical analysis was performed using a generalized linear model.
ELS = embryo-like structures per 100 anthers
tELS = transferred ELS per 100 anthers
Green = regenerated green plants per 100 anthers
Green / tELS = percentage of greens per tELS
Alb = albino plants regenerated per 100 anthers
Tot = sum of the green and alb per 100 anthers
Tot / tELS = percentage of Tot from tELS
Greens / Tot = percentage of green of Tot

over-representation could not be statistically assessed, since the expected segregation ratio of the marker in DH regenerants could not be determined due to the sample structure of the anther-donor plant material (Table 1).

In conclusion, a recalcitrant oat genotype was crossed with a responsive wild red oat genotype, which produced cross plants with significantly better anther culture ability traits. However, in further backcrosses these traits were lost rapidly. A fast and efficient RAPD method was used to screen lines from the cross and backcross progenies in order to select responsive genotypes. Two putative RAPD markers associated with improved anther culture traits of oats, one (OPX-11_{870}) derived from acc. CAV 2648 and one (OPC-10_{480}) from cv. Puhti, are proposed in this study. These markers might be used for selecting lines carrying alleles that result in better doubled haploid production from cultivated and wild red oat cross and backcross progenies. To increase the reproducibility of markers, they could be converted into SCAR markers, which are less sensitive to reaction conditions. In addition, the putative trait – marker associations found in this study should be verified prior to practical applications.

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SELOSTUS

Ponsiviljeltävyys ja siihen liittyvät geenimerkit peltokauran ja susikauran risteytysjälkeläisissä

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Ponsiviljelyn avulla voidaan indusoida kypsymättömistä siitepölyhiukkasista täysin homotsygoottisia kaksoishaploideja kasveja yhden sukupolven aikana. Tehokas haploidiajalostus hyödyntää tätä mahdollisuutta tuottaa vaihtelevista risteytysjälkeläisistä nopeasti täysin puhtaita linjoja. Kaksoishaploideja kasveja käytetään myös ominaisuuksien periytymisen tutkimiseen. Ponsiviljelyä rajoittaa kuitenkin sen onnistumisen vahva riippuvuus genotyypistä. Tätä ominaisuutta säätelevät useat riippumattomasti periytyvät geenit, jotka vaikuttavat prosessin eri vaiheissa määräten alkiorakenteiden induktiota, kasvien regeneroitumista sekä vihreiden ja albiinojen kasvien suhdetta. Kauran heksaploidin villin sukulaisen, susikauran (*Avena sterilis* L.) linja CAV 2648, on kokeissamme aiemmin tuottanut toistettavasti kaksoishaploideja kasveja. Kun ponsiviljelyssä täysin reagoimaton peltokauran (*Avena sativa* L.) lajike Puhti risteytettiin CAV 2648 linjan kanssa, vihreiden kasvien regeneroiminen jälkeläisistä onnistui. Tässä työssä tutkittiin mahdollisuutta tuoda susikaurasta peltokauraan ponsiviljelyä parantavia alleeleja risteytyksen ja kahden takaisinristeytyksen avulla. Lisäksi etsittiin ponsiviljelyyn kytkeytyneitä RAPD-geenimerkkejä, joiden avulla vaihtelevista risteytysjälkeläisistä voitaisiin tulevaisuudessa valita paremman ponsiviljeltävyyden omaavat linjat.

Genotyypin havaittiin vaikuttavan kaikkiin testattuihin ponsiviljelyominaisuuksiin, joita olivat alkiorakenteiden induktio sekä vihreiden ja albiinojen kasvien erilaistuminen suhteessa ponsien määrään ja erilaistumisalustalle siirrostettujen alkiorakenteiden määrään. Eniten alkiorakenteita saatiin CAV 2648 linjasta, mutta vihreiden taimien regeneroituminen oli paras Puhti × CAV 2648 F_2 risteytysjälkeläisistä. Ponsiviljelyn onnistuminen huononi nopeasti takaisinristeytyksissä, ja Puhti-lajike oli odotettavasti kaikkien mitattujen ominaisuuksien suhteen huonoin.

Vanhempaisgenotyypit Puhti ja CAV 2648 testattiin 186 RAPD-alukkeella, joista 17 antoi elektroforeesigeelissä vanhempien välillä selvästi eriävän bändikuvion. Näiden 17 alukkeen tuottamat 53 geenimerkkiä testattiin 38:lla Puhti × CAV 2648 F_2 kasvilla ja saatua dataa verrattiin samoista kasveista saatuun ponsiviljelydataan (jokaisesta kasvista 90 eristetystä ponnesta saadut ponsiviljelytulokset). Tilastoanalyysien perusteella neljän alukkeen tuottamat viisi geenimerkkiä olivat kytkeytyneet mitattuihin ponsiviljelyominaisuuksiin. Näistä OPX-11_{870} -merkki oli kytkeytynyt vihreiden sekä albiinojen regeneranttien tuottoon eristetyistä ponsista ja OPC-10_{480} -merkki oli kytkeytynyt sekä vihreiden että kaikkien regeneroitujen kasvien yhteenlaskettuun tuottoon suhteessa sekä eristettyihin ponsiin että siirrostettuihin alkiorakenteisiin. Näistä merkeistä ensin mainittu oli peräisin CAV 2648 linjasta ja toinen yllättäen heikon ponsiviljelyvasteen omaavasta Puhti-lajikkeesta. Kirjallisuuden perusteella tämä ei ole tavatonta, vaan positiivisesti vaikuttavia alleeleja voi periytyä myös huonomman vasteen omaavasta genotyypistä, koska ponsiviljelyvaste on monimutkaisesti säädelty ominaisuus. Lisäksi löydettiin kolme CAV 2648 linjasta periytyvää merkkiä, jotka olivat kytkeytyneet alhaisiin induktio- ja regeneraatiotasoihin.

Yhteenvetona voidaan todeta, että risteyttämällä peltokaura paremmat ponsiviljelyominaisuudet omaavan susikauran kanssa, jälkeläisten kaksoishaploidituotto paranee verrattuna huonompaan vanhempaan. Takaisinristeytyksissä peltokauraan ominaisuus häviää kuitenkin nopeasti. Tutkimuksessa löydettyjä geenimerkkejä käyttäen voitaisiin risteytysjälkeläisistä valita jatkoon niitä genotyyppejä, joilla on perimässään parempiin ponsiviljelyominaisuuksiin johtavia alleeleja.