Phenotypic characterization and bulk segregant analysis of anther culture response in two backcross families of diploid potato

RAPD markers for androgenesis in potato

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Abstract

Two diploid (2n=2x=24) backcross potato populations (PBCp, and CBC) were characterized for anther culture response (ACR). PBCp (Solanum phureja Juz. & Buk. genotype 1-3 × CP2) and CBC (CP2 × S. chacoense Bitt. genotype 80-1) resulted from a cross between CP2 (intermediate ACR) and its parents, S. chacoense 80-1 (low ACR) and S. phureja 1-3 (high ACR). Three components of ACR were initially investigated: embryos per anther (EPA), embryo regeneration rate and percent monoploids (2n=1x=12) among regenerants. EPA was selected for further characterization because of its relative stability. In a series of studies of EPA on a total of 44 genotypes within CBC, nine high (mean EPA=2.5) and ten low (mean EPA=0.02) selections were made. In PBCp, ten high (mean EPA= 4.7) and ten low (mean EPA= 0.05) selections were made from 67 genotypes. High and low selections were used for bulk segregant analysis to screen 214 RAPD primers as candidate markers linked to EPA. Bands amplified by OPQ-10 and OPZ-4 were associated in coupling and repulsion, respectively, to ACR in PBCp. A band amplified by OPW-14 primer was associated in coupling to ACR in CBC. One-way ANOV As using presence/absence of each candidate band to classify additional genotypes in each population verified association of the markers with EPA.

Abbreviations: ACR – anther culture response; EPA – embryos per anther; CBC – backcross between CP2 and S. chacoense 80-1; PBCp – backcross between S. phureja 1-3 and CP2

Introduction

In potato, anther culture response has been found primarily in a few genotypes that are commercially unimportant. A limitation in potato breeding has been the unavailability of homozygous lines, because of self-incompatibility of most diploids (2n=2x=24) and in-breeding depression exhibited after selfing most genotypes, when possible, regardless of ploidy. Genetic maps of potato have been derived from compromised populations with unpredictable segregation ratios (Gebhardt et al., 1991; Tanksley et al., 1992; Jacobs et al., 1995; Hosaka, 1999). Doubled-haploids developed by anther culture can be used as inbred lines in mapping or in breeding programs (Wenzel et al., 1979). However, for this to occur, potato clones must be competent for androgenesis. Dihaploid extraction by anther culture of superior tetraploid (2n=4x=48) clones has been limited (Rokka et al., 1996); therefore, transferring this ability to commercially desirable clones or enhancing it would facilitate potato breeding at the diploid level.

contrast, Sonnino et al. (1989) suggested that several recessive genes controlled anther culture response, as measured by embryos per anther (EPA). Later, Meyer et al. (1993) proposed the involvement of no more than two genes for ACR. However, Singsit and Veilleux (1989) found no positive correlation between embryo formation and regeneration of embryos into plantlets, thus suggesting that these two characters were independently inherited.

There have been several attempts to tag genes associated with ACR in several crops. In maize (Zea mays L.), six chromosomal regions associated with either formation of embryo-like structures (ELS) or regenerable callus were identified by Wan et al. (1992). Murigneux et al. (1994) identified three to four quantitative trait loci (QTL) involved in ACR. Beaumont et al. (1995) identified six chromosomal regions associated with the induction of embryo-like structures from microspores. In barley (Hordeum vulgare L.), Devaux and Zivy (1994) identified molecular markers associated with ACR linked either to genes involved in both embryo production and green plant regeneration or only to genes involved in green plant regeneration. Komatsuda et al. (1995) located a gene (ShdI) in a parental line of barley for shoot differentiation of immature embryo-derived callus. Manninen (2000) identified ten markers associated with three components of anther culture (percent responding anthers, plants per responsive anther and percent diploid green plants) among doubled haploids derived from an F1 progeny of barley. In rice (Oryza sativa L.), He et al. (1998) identified five QTLs for callus induction frequency and one major QTL for albino plantlet differentiation frequency. In oilseed rape (Brassica napus L.), Cloutier et al. (1995) identified two linkage groups as putative chromosomal regions associated with microspore-culture responsiveness. In contrast to potato, inbred lines are available for all these crops, making it less complicated to tag anther culture response.

Cloning genes that control ACR may facilitate their integration by gene transfer techniques into desirable potato genotypes. This research represents a step in this process. In this study, our objectives were to characterize the components of ACR in two backcross populations of diploid potato segregating for this trait and to identify randomly amplified polymorphic DNA (RAPD) markers linked to this trait using a bulk segregant analysis (BSA); a technique that consists of pooling DNA of genotypes exhibiting extreme phenotypes of a trait in a segregating population (Michelmore et al., 1991).

Materials and methods

Plant material

Two backcross populations (PBCp and CBC) were established by crossing a self-incompatible interspecific hybrid (CP2) resulting from a cross between genotypes of S. chacoense (chc) and S. phureja (phu) back to its parents as follows: phu 1-3 × CP2 → PBCp and CP2 × chc 80-1 → CBC. In preliminary experiments, anther culture response (ACR) was low for chc 80-1, high for phu 1-3, and intermediate for CP2. Plants were grown in the greenhouse under 16-h photoperiod and 25–30 °C day/15–20 °C night. The photoperiod was extended to 16 h when needed, using halogen lamps (1000 watts). For PBCp, 67 genotypes were characterized whereas 44 genotypes were characterized for CBC.

Culture technique

Flower buds containing microspores at late-uninucleate to early-binucleate stages were collected and placed in a refrigerator at 4 °C for 3 days. Buds were surface-sterilized by immersion for 1 min in 80% ethanol, then 5 min in 100% household bleach with 2 drops of ‘Tween 20’, and finally rinsed twice in sterile-distilled water. Flower buds were dissected, and 30 anthers were placed in a 125 ml culture flask containing 15 ml liquid medium [1/2-strength Linsmaier and Skoog (1965) basal salts supplemented with 100 mg l⁻¹ myo-inositol, 0.4 mg l⁻¹ thiamine, 6% sucrose, 2.5 g l⁻¹ activated charcoal, 2.5 mg l⁻¹ N6-benzyladenine, and 0.1 mg l⁻¹ indole-3-acetic acid (IAA), pH 5.8]. A total of ten flasks per genotype was used whenever possible, based on availability of buds, flasks were sealed.