Australian Journal of Crop Science
1(2):43-46 (2008)
ISSN: 1835-2707

Southern Cross Journals© 2008
www.cropsciencejournal.org

Identification of markers linked to agronomic traits in globe artichoke

Eugenia Martin[1], Vanina Cravero[1*], Andrea Espósito[2], Fernando López Anido[2], Leonardo Milanesi[2] and Enrique Cointry[2].

[1] *CONICET, Zavalla, Santa Fe, Argentina.*
[2] *Genetics and Plant Breeding, Universidad Nacional de Rosario, Zavalla, Santa Fe, Argentina.*
**Corresponding author email: vcravero@unr.edu.al*

Abstract

Color head and precocity of production are two important agronomic traits in globe artichoke which determine the acceptance of the product in the market. 118 F_2 plants were analyzed for two agronomic traits, color head and precocity of production. Bulked Segregant Analysis (BSA) and Sequence-related amplified polymorphism (SRAP) were used to detect molecular markers linked to the traits. Ten plants with the same trait were used to constitute the bulks, which were screened with seven SRAP pair primers. We detected one band/marker that may be linked to the green color head and one band/marker that may be associated with precocity in head production. The results obtained show that the BSA and SRAP analyses are useful to identify molecular markers associated with agronomic traits. The identified markers will potentially be used in molecular marker aided-selection (MAS) for breeding programs in globe artichoke.

Keywords: Globe artichoke, Molecular markers, SRAP, BSA, MAS.

Introduction

Globe artichoke (*Cynara cardunculus* L. var. *scolymus*) (2n=2x=34) is a perennial and cross-pollinated vegetable (Pécaut, 1993) native of the Mediterranean basin (Rottenberg and Zohary, 1995). Artichoke heads or capitulum, which are immature composite inflorescences, are the edible parts of the plant and are used as a fresh, frozen or canned delicacy all over the world. Color head and precocity of production are two important agronomic traits which determine the acceptance of the product in the market. The attainment of precocious cultivars can increase the profit because the production can enter the market at the best prices time, while late cultivars generate productions that can be exported to northern countries. Artichoke breeding to date has been limited to a few studies on the inheritance of a small number of main traits such as precocity, color head, spiny and compacity (Pécaut, 1993; Lòpez Anido *et al*, 1998 Mauromicale *et al*, 2000; Cravero *et al,* 2005). Molecular marker studies of the artichoke genome have also been limited to germplasm characterization (Tivang *et al.,* 1996*;* Sonnante *et al.,* 2002*;* Lanteri *et al.*, 2004; Acquadro *et al.*, 2005 and Cravero *et al.*, 2007). Only Lanteri *et al.* (2006) reported two SSR markers linked to spiny bracts. Sequence-related amplified polymorphism (SRAP) was recognized as a new and useful molecular marker system for mapping and gene tagging in *Brassica oleracea* L. (Li and Quiros, 2001) and other crops such as rice, lettuce, garlic, etc (Li and Quiros, 2001). These markers are more consistent and repeatable than RAPDs, and less labour-intensive and time consuming than AFLP technique (Li and Quiros, 2001; Ferriol *et al.*, 2003; Budak *et al.,* 2004). Bulked Segregant Analysis (BSA) provides a rapid, technically simple alternative for identifying markers linked to specific genes (Michelmore *et al.*, 1991). BSA compares two pooled DNA samples of individuals from a segregating population, which contrast for a trait.

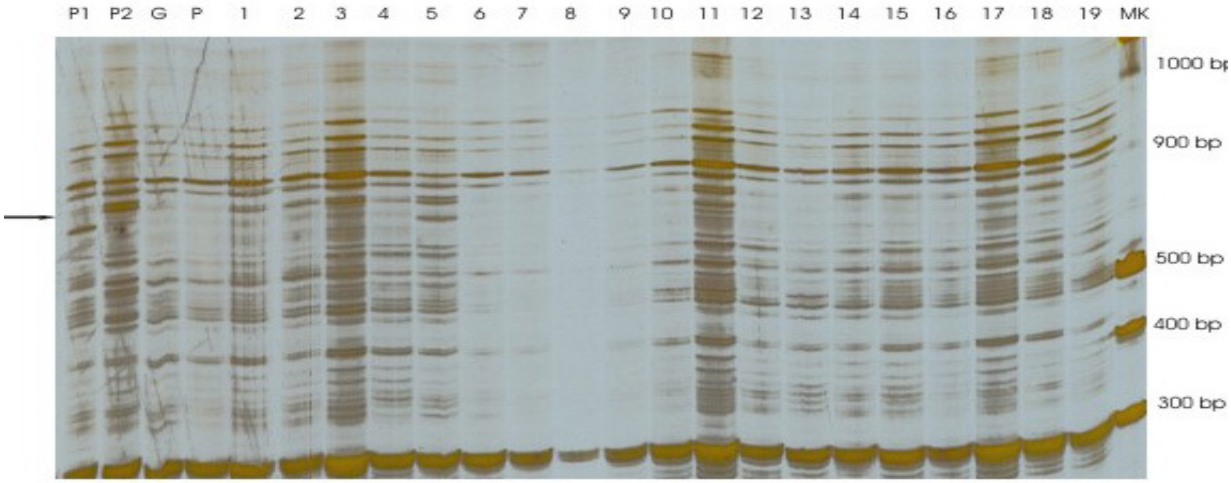

Fig 1. Marker linked to head color. The arrow indicated the polymorphism band between Parents Bulks (P1 and P2), between F_2 Bulks (G: Green and P: Purple), and individuals F_2: 1-10 are green bulk and 11-19 are purple bulk. MK: Ladder Perfect DNA 50 bp (NOVAGEN).

The aim of the present work was to identify markers linked to color head and precocity of production using SRAP markers and BSA.

Materials and Methods

A controlled cross between two varietal types of *C. cardunculus* var. *scolymus* was performed to get the F_1 and F_2 generations. Estrella del Sur FCA variety (P1, used as female parent) has late-production and purple heads, whereas Imperial Star (P2, used as male parent) has early-production and green heads. Both parents and F_2 generation were seeded in speedling and transplanted at two true leaves stage. Plants were implanted at the Experimental Station of the Rosario National University placed in Zavalla (Argentina). Comercial maturity stage becames between October to November. At this stage, all F_2 individuals were harvested and morphologically characterized for color head and precocity. For the first trait, three categories were established: purple, green and variegate (purple-green) color. Precocity was evaluated as days required from seedling to main head harvest; "late-production" included all plants that require more than 180 days to begin production, and "early-poduction" included those plants that require less than 160 days. Plants which require between 160 and 180 days from seedling to harvest were discarded. Genomic DNA was extracted from fresh leaves of each plant using the PureLink™ Plant Total DNA Purification Kit (Invitrogen Carlsbad – California). DNA concentration was estimated in 8 % (w/v)agarose-gel stained with ethidium bromide in comparison with a λ DNA standard. Each bulk was performed with DNA from ten F_2 plants with the same characteristic in equal quantity. Four bulks were obtained: "purple-head", "green-head", "late-production" and "early-production". Seven different primers combinations, which generated high level of polymorphisms in *Cynara cardunculus* (Cravero *et al.,* 2007), were screened on both parents and on the bulks (table 1 a and b). Each 20 µl PCR reaction mixture consisted of 20 ng of genomic DNA, 5 mM dNTPs, 50 Mm $MgCl_2$, 10 µM of each primer, 5.2 µL 10X Taq buffer and 4 units of Taq polymerase. Samples were subjected to the following thermal profile: 5 min of denaturing at 94°C, 5 cycles of three steps: 1 min of denaturing at 94°C, 1 min of annealing at 35°C and 1 min of elongation at 72°C, for the next 35 cycles annealing temperature was elevated to 50°C, ending with elongation step of 10 min at 72°C. Separation of amplified fragments was accomplished on 6% (w/v) denaturing polyacrylamide gels at 60 W for 4 hours at room temperature. Gels were stained with $AgNO_2$. SRAP markers were scored for presence (1) or absence (0) of a band.

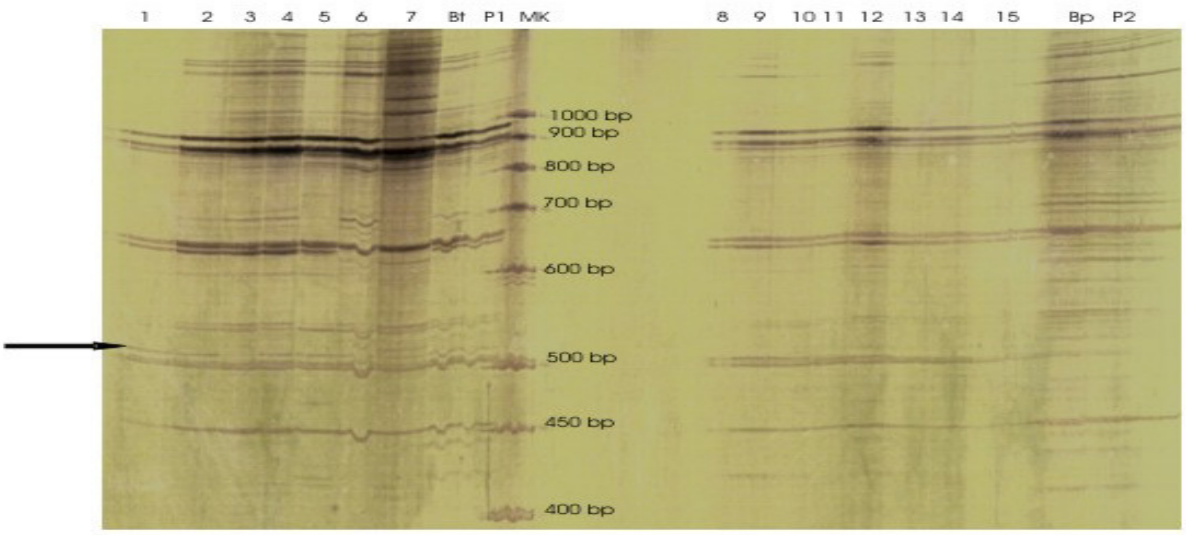

Fig 2. Marker linked to precocity. The arrow indicated the polymorphism band between Parents Bulks (P1 and P2), between F_2 Bulks (Bt: Later-production and Bp: Early-production) and individual F_2: 1-7 late production bulk and 8-15 early production bulk MK: Ladder Perfect DNA 50 bp (NOVAGEN).

Table 1 a. Sequence of primers used for SRAP

Primer sequences	
Forward	**Reverse**
Me1: TGAGTCCAAACCGGATA	Em2:GACTGCGTACGAATTTGC
Me2: TGAGTCCAAACCGGAGC	Em3:GACTGCGTACGAATTGAC
Me3: TGAGTCCAAACCGGAAT	Em4:GACTGCGTACGAATTTGA
Me4: TGAGTCCAAACCGGACC	Em5:GACTGCGTACGAATTAAC

Table 1 b. Combinations of primers used for SRAP

Primer combinations						
1	**2**	**3**	**4**	**5**	**6**	**7**
Me1 – Em4	Me1 – Em5	Me2 – Em4	Me3 – Em2	Me3 – Em 5	Me4 – Em3	Me4 – Em4

Results and Discussion

Out of 118 F_2 plants were obtained, 30 had purple heads, 23 had green heads and 65 had variegated ones. All segregated as expected with a ratio of 9:3:4 ($\chi^2 = 4.6$; $p<0.05$) for a trait controlled by two major genes with simple recessive epistasis (Cravero *et al.*, 2005). At harvest time, precocity was also evaluated, 12 F_2 plants required less than 160 days to begin production, 13 plants required more than 180 days and 94 plants showed intermediate precocity (between 160 and 180 days).

By using BSA strategy, we were able to detect one band/marker presumptively linked to color head. This band was generated by the primer combination Me4-Em4 and is approximately 850 bp. It´s in coupling phase to the green color and was detected in the Imperial Start parent (P2) and in the green bulk (Fig. 1). With the primer combination Me3-Em5, we detected a polymorphic band between "early" and "late-production" bulks. This band is about 520 bp, which can be detected in Estrella de-

l Sur FCA (P1) and the "late-production" bulk but absent in the other parent and bulk (Fig. 2).

The putative markers were tested in the individual F_2 plants included in each bulk. Not all individuals showed a good banding pattern, so that only fifteen plants could screen for color head (five green and seven purple) and fifteen for precocity (seven late-production and eight early). The marker that cosegregates with the green head phenotype was present in all green heads plants and absent in all purple head ones. The band associated to precocity appeared in six of the seven plants included in "late-production" bulk and it was absent in all plants corresponding to the "early" bulk.

In conclusion, this study has identified putative SRAP markers linked to two important agronomic traits in globe artichoke: head color and precocity of production. However, the degree of association between those SRAP markers and the respective agronomic traits need to be investigated by means of the screening in the whole F_2 population. If this association is confirmed these markers will be useful for globe artichoke breeding, which lacks a well-developed molecular map. This is the first report of a potentially useful gene tagging in globe artichoke using SRAPs.

References

Acquadro A, Portis E, Lee D, Donini P and Lanteri S (2005) Development and characterization of microsatellite markers in Cynara cardunculus L. Genome 48:217-225

Budak H, Shearman RC, Parmaksiz I, Gaussoin RE, Riosdan TP and Dweikat I (2004) Molecular characterization of buffalograss germplasm using sequence-related amplified polymorphism markers. Theor Appl Genet 108:328-334

Cravero V, Martin E and Cointry E (2007) Genetic diversity in *Cynara cardunculus* determined by Sequence-related Amplified Polymorphism Markers. J Amer Soc Hort Sci 132:1-5

Cravero V, Picardi L and Cointry E (2005) An approach for understanding the heredity of two quality traits (head color and tightness) in globe artichoke (*Cynara scolymus* L.). Genet Mol Biol 28:431-434

Ferriol M, Picó B and Nuez F (2003) Genectic diversity of a germplasm collection of *Curcubita pepo* using SRAP and AFLP markers. Theor Appl Genet 107:271-282

Lanteri S, Acquadro A, Comino C and Mauro R (2006) A first linkage map of globe artichoke (*Cynara cardunculus* var. *scolymus* L.) based on AFLP, S-SAP, M-AFLP and microsatellite markers. Theor Appl Genet 112:1532-1542

Lanteri S, Saba E, Cadinu M, Mallica GM, Baghino L and Portis E (2004) Amplified fragment length polymorphism for genetic diversity assessment in globe artichoke. Theor Appl Genet 108:1534-1544

Li G and Quiros C (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: Its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103:455-461

López Anido F, Firpo I, García S and Cointry E (1998) Estimation of genetic parameters for yield traits in globe artichoke (*Cynara scolymus* L.). Euphytica 103:61-66

Mauromicale G, Morillo N, Santoiemma G and Ierna G (2000) Nouve varietà per megliorare la inaricoltura siciliana. Inf Agrario 26:47-51

Michelmore R, Paran I and Kesseli R (1991) Identification of markers linke to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828-9832

Pécaut P (1993) Globe artichoke *Cynara scolymus* L. In: Kallo G., Bergh Bd (eds) Genetics improvements of vegetable crops, Pergamon, Oxfords, 746-767

Rottenberg A and Zohary D (1995) The wild relatives and the wild ancestry of the cultivated artichoke. Genet Resour Crop Evol 43:53-58

Sonnante G, De Paolis D, Lattanzio V and Perrino P (2002) Genetic variation in wild and cultivated artichoke revealed by RAPD markers. Genet Resources Crop Evol 49:247-252

Tivang J, Skroch P, Nienhuis J and De Vos N (1996) Randomly Amplified Polymorphic DNA (RAPD) variation among and within artichoke (*Cynara scolymus* L.) cultivars and breeding populations. J Amer Hort Sci 121:783-788