CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

An integrated model to accelerate the development of seed-propagated varieties of globe artichoke

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Abstract: *Globe artichoke (Cynara cardunculus var. scolymus) is a cross-pollinated, highly heterozygous species, which is conventionally propagated vegetatively. A scheme is described here which combines phenotypic with genotypic selection to fast track the development of a seed-propagated variety. The scheme was tested by making three selections, on a phenotypic basis, from a Brazilian seed-propagated variety showing an high phenotypic variation. The genetic relatedness as well as the heterozygosity of the material in study, in respect to standard variety representatives, was initially assessed with a wide set of microsatellite markers. Afterwards, an AFLP-based selection demonstrated to provide a practical and cheap means of conducting marker assisted breeding, which can be easily adopted also in laboratories of small seed companies. The selection approach described here could be readily adopted also to convert current vegetatively propagated landraces into seed-propagated varieties.*

Key words*: AFLP, Cynara cardunculus, seed propagated cultivars, SSR.*

INTRODUCTION

The species *Cynara cardunculus* L. [*Compositae* (a.k.a. *Asteraceae*), 2n = 2x = 34] includes three botanical taxa: the globe artichoke (var. *scolymus* L.), the cultivated cardoon (var. *altilis* DC.) and the wild cardoon [var. *sylvestris* (Lamk) Fiori]. Globe artichoke, which harbours a highly heterozygous genetic background (Scaglione et al. 2009), is cropped largely in the Mediterranean region for its immature inflorescence, referred to as the capitulum or heads (Pandino et al. 2011), whose inner bracts and fleshy receptacle are consumed as fresh, preserved and frozen delicacy. The leading world producer is Italy but its cultivation has recently spread to the Americas and China, and its production has risen from about 1.2 Mt in 1994 to 1.6 Mt in 2014 (http://faostat.fao.org/). Italy is also thought to be the globe artichoke centre of domestication (Mauro et al. 2009).

The more than 100 varietal types cultivated (Mauromicale and Ierna 2000) are divided, on the basis of their harvest time, into 'reflowering types' (which flower between autumn and spring), and 'non-reflowering types' (which flower only during spring). A further distinction between types is based on a variety of capitulum traits such as dimension, shape, presence/absence of spines, and pigmentation of the outer bracts (Basnitzki and Zohary 1994, Lanteri and Portis 2008). The crop, represented by a small number of varietal types and many local landraces, has long been - and continues to be - largely propagated

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vegetatively by means of basal and lateral offshoots (either semi-dormant or actively growing). The latter guarantees the maintenance over time of the desired traits, but it is responsible of disadvantages like physiological heterogeneity of the propagative material, diffusion of pathogens (mainly viruses), low rate of multiplication and flexibility in transplant schedule, high cost for plantation and high percentage of planting failures (Mauromicale et al. 2000). The development of efficient protocols of *in vitro* multiplication has solved many of the technical problems associated with traditional propagation methods, but they are very expensive and do not lend themselves well to some varietal types (Saccardo et al. 2013). Meanwhile, seed-propagated cultivars have grown in popularity as they allow the crop to be treated as an annual, reduce the cost of planting, the diffusion of pathogens as well as the use of fertilizers and needs of watering, since plants develop a deeper root systems better exploiting water and nutrients. Significantly, seed-propagation allows new varietal types to be both developed and diffused more rapidly than vegetatively-propagated ones.

Some of the seed-propagated cultivars currently available are open pollinated varieties, while most are F_1 hybrids. The former rely on the intercrossing of a set of partially inbred selections, and can suffer a degree of pollen contamination, which can lead to the appearance of undesirable phenotypes. F_1 hybrid seed is produced by exploiting male sterility; but since the species does not tolerate extensive inbreeding, commercial F_1 hybrids are not quite as uniform as experimental ones obtained from crosses between two highly homozygous inbreds. Nevertheless, despite the high cost of their seed, hybrids are increasing in popularity thanks to their predictably high yields.

Here, a selection scheme is presented which combines mass phenotypic selection, self-pollination and marker assisted selection, starting from a population of the seed- propagated and open pollinated Brazilian variety 'Nobre UPF'. The goal was to identify a small number of individuals which, when intercrossed, could guarantee both a high and a stable level of production. The scheme is readily applicable to developing an open pollinated variety from vegetatively propagated landrace material, the survival of which is threatened by the spread of F_1 hybrids.

MATERIAL AND METHODS

Plant material and experimental setup

A set of 200 seeds of the Brazilian cultivar 'Nobre UPF' (Baggio et al. 2011) was sown in September 2011 in an experimental field at the University of Catania (lat 37° 03' N, long 15° 18 E, alt 10 m asl), Sicily, Italy. The local climate provides mild, wet winters and warm, dry summers. Within each row, each plant was separated from its neighbour by 0.80 m and the inter-row spacing was 1.25 m; the result planting density was one plant per m^2. A pre-sowing dressing of 180 kg ha^{-1} P_2O_5 and 140 kg ha^{-1} K_2O was given, and three applications of 70 kg ha^{-1} N were provided in September, November and February. The crop was drip-irrigated between sowing until mid-October and from May to middle July, all the experimental plots were kept weed and insect-free by spraying oxyfluorfen and imidachoprid, respectively, when required. In the following spring, three phenotypic groups, denoted NP_2, NP_4 and NP_5, were identified on the basis of the number of floral stem ramifications (an index of yield potential) present and capitulum shape and thickness (Figure 1). NP2 plants produced compact and sub-globular capitula, from 4 to 5 floral stems, and the height of the stem of the main capitulum was about 85 cm; NP4 plants produced compact and spherical capitula, from 3 to 4 floral stems, and the average height of the main stem was 100 cm; NP5 produced oblong medium-sized capitula, from 5 to 6 floral stems, and the height of the main stem was about 95 cm. A representative individual from each group was propagated from rhizomes bearing quiescent buds ("ovoli"), and the resulting clones (14 per group) were cultivated as described above. In late spring 2013, seven plants per group were isolated within an insect-proof cage just before anthesis, and a hive housing about 300 *Bombus terrestris* bumble bees was placed within each of the three cages to maximize inter-crossing. In parallel, at least three plants per group were allowed to out-pollinate by growing them uncaged. Both groups of plants were phenotyped for plant traits, scored for fertility, certain seed related traits and fully mature achenes were collected towards the end of July 2013. A further three- four plants per group, out of the cage, other than for plant traits were evaluated for the production of immature inflorescences, which were collected and weighted at the commercial stage.

The seeds (achenes) harvested from cage isolated plants of each clone (selfed progeny) were sown in September 2013, and a group of 14-16 plants per group were phenotypically selected for cultivation during 2013-2014. These plants phenotyped, scored for fertility, certain seed-related traits and seed production. Capitulum weight at the commercial harvest stage was not assessed as the capitula were left on the plants to allow seed set. AFLP profiles were generated

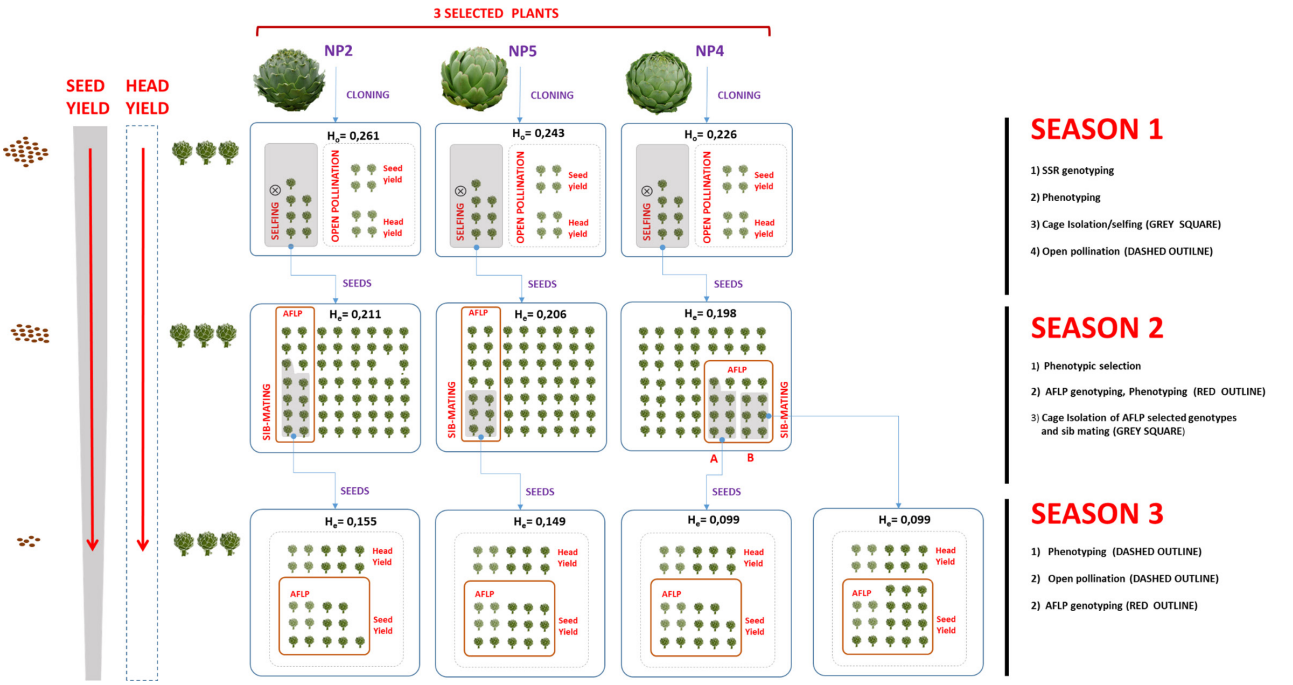

Figure 1. A schematization of the adopted selection program.

from each individual and used to define sets of closely related plants; these were once again isolated within a cage in presence of *Bombus terrestris* as described above. Seed was collected in late July 2014. In September 2014 a randomly chosen sample of seeds (achenes) was sown and a sample of 13-16 plants per group was phenotyped (see below), assessed for fertility and the seed-related traits, and re-profiled by AFLP. A parallel sample of ten randomly chosen plants per group was tested for the weight of the immature inflorescences at the commercial stage. A schematization of the adopted selection program is shown in Figure 1.

Phenotypic characterization

The number of capitula per plant, pollen viability, the number and weight of achenes per plant and the weight of 1,000 achenes produced by the main and first order capitula were assessed in each of the three seasons; a fruit setting index (FSI) was calculated from the ratio between the number of achenes per plant and the number of flowers per plant.

Pollen viability was assessed at the microscope after staining pollen grains with 2% w/v acetic carmine. The pollen viability was scored according to staining level (pollen with bold red colour as viable and colourless as nonviable). The percentage of pollen viability was determined as the ratio of the number of viable grains to the total grains number'. A petri dish-based germination test was conducted on four replicate lots of 50 seeds placed in an incubator in the dark at the temperature of 18 ± 1 °C; both the germination percentage and the mean germination time were calculated: seeds were considered as germinated when the radicle had reached a length of 1 mm, and the mean germination time was given by the expression $\frac{\Sigma nd}{N}$, where *n* is the number of germinated seeds on each day, d the number of days from the beginning of the test and N the total number of germinated seeds. In each season, at the head harvestable stage (length of the central global flower buds < 2 mm), the following other plant traits were measured: plant height (measured from soil surface to the plant apex), the length and maximum diameter of the floral stem bearing the main, the maximum length and width of the 24th, 25th and 26th leaf and the weight of capitula. The variances were checked for homogeneity using the Bartlett test, and the data subjected to an analysis of variance in which the main effects were genotype and season. Means were discriminated on the basis of Fisher's protected least significant difference (LSD).

Genotypic characterization

DNA was extracted from young leaves following the Lanteri et al. (2004) protocol. Microsatellite (SSR) fingerprints, based on 125 loci distributed across all 17 linkage groups (Scaglione et al. 2009), were obtained from a single representative plant from each of the three phenotypic groups NP_2, NP_4 and NP_5, as well as from a single plant of 12 cultivars (Spinoso di Palermo', 'Violetto di Toscana', 'Spinoso Violetto di Liguria', 'Empolese', 'Pietralcina', 'Romanesco C3, 'Pasquaiolo', 'Green Globe', 'Violetto di Provenza', 'Sakiz', 'AVM' and 'Blanco') maintained in the University of Catania's living germplasm collection. The SSR fingerprinting was carried out using the protocol reported by Scaglione et al. (2009).

AFLP fingerprinting was carried out using the Lanteri et al. (2004) protocol. Each amplified fragment in the size range 60–650 bp was assumed to represent a single bi-allelic locus, in order to generate a presence/absence-based binary genotypic matrix. Genetic similarities between pairs of individuals were quantified via the Jaccard (1908) similarity index, which provided the basis for both constructing a UPGMA-based dendrogram and conducting a principal coordinate analysis (PCoA). The polymorphic information content (PIC) was calculated following Anderson et al. (1993)

RESULTS AND DISCUSSION

F_1 hybrid globe artichoke varieties have been increasing their market share in spite of the high cost of their seed, since they are perceived to be higher yielding than the conventional varieties. This perception has been borne out by the outcome of a number of controlled experiments (Mauromicale and Ierna 1995, Calabrese et al. 2005, Rey et al. 2013). On the other hand, their capitula contain lower amounts of polyphenol and develop thicker external bracts, which decrease their nutritional value and their market attractiveness (Bonasia et al. 2010). Meanwhile their steady replacement in farmers' fields of the long-established local varieties, which have a long history of selection for organoleptic quality and local adaptation, is fast eroding the genetic base of the crop.

Commercial F_1 hybrids are not as uniform as experimental ones produced by crossing a pair of highly inbred lines, and genotypic analyses have confirmed the hybrids are quite heterogeneous at the genetic level (unpublished data). A potential alternative approach to developing a seed-propagated variety sufficiently distinct, uniform and stable at the phenotypic level, thus satisfying the regulatory requirements for varietal release, could be to generate an open pollinated variety bred from a group of closely related progenitors. As yet there is no firm understanding as to what level of homozygosity can be tolerated in globe artichoke before plant vigour and/or the yield or capitulum quality are compromised.

Genetic variability and genetic relatedness

Of the 125 SSR assays applied to the NP and standard variety representatives, 115 produced scorable amplicons. The SSR-derived phylogenetic analysis of the 15 genotypes (Figure 2A) showed that the three NP selections were well differentiated from the reference varietal types. The latters' mean level of heterozygosity was ~58% (ranging from 46% in 'Pasquaiolo' to 70% in 'Romanesco C3'), and was surprisingly high also in the seed-propagated reference variety 'Green Globe'. On the other hand the heterozygosity level in the three NP selections was much lower (respectively, 26, 23 and 24%) (Figure 2B).

The AFLP fingerprint of the selfed generation of the NP

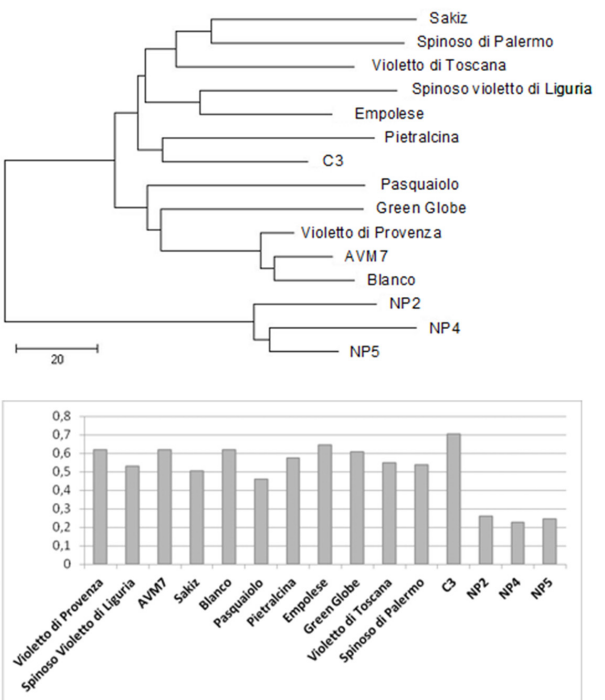

Figure 2. Genetic relatedness between the three NP selections and the 12 reference varieties. A) A UPGMA cluster analysis (Nei-Li's genetic similarity) and B) percentage heterozygosity values, based on the allelic status at 115 SSR loci.

selections (Figure 3A) confirmed that the initial selections were genetically distinct, as their progenies were grouped into three main clusters. Within each main cluster, two sub-clusters as well as four outliers were identified: NP_5_12, NP_4_1, NP_4_10 and NP_4_11; these individuals lay outside the clade containing their sibs. As the aim was to promote genetic uniformity within each of the three NP groups, a group of nine individuals from NP_2 and six from NP_5 were retained; because the NP_4 was more heterogeneous, two sub-groups (NP_{4A} [seven plants] and NP_{4B} [six plants]) were carried forward for the sib-mating generation. In the PCoA conducted on the progeny prior to sib-mating (Figure 3B), the first two principal axes accounted for, respectively, ~48% and ~26% of the genetic variance. Axis 1 distinguished NP_2 and NP_5 progenies from those of both NP_4 ones, as well as those in NP_{4A} from those in NP_{4B}, while Axis 2 replicated the three cluster structure predicted by the earlier analysis. Both axes confirmed that the NP_2 and NP_5 progenies were more variable than the NP_{4A} and NP_{4B} ones. In the PCoA derived from the set of AFLP fingerprints acquired from the progeny of the sib-mating (Figure 3C), the first two principal axes accounted for, respectively, ~70% and ~25% of the genetic variance. The cluster structure was compatible with that seen in the previous generation. As was the case in the earlier generation, the members of NP_{4A} and NP_{4B} were more genetically uniform than those in the other two groups, to the point where some of the individuals appeared to be genetically almost identical. The PIC values obtained from AFLP profiling and associated with each individual genotyped in the second and third seasons are shown in Figure 1.

With reference to their progenitor, the first round of selfing decreased heterozygosity from about 26% to 21% in

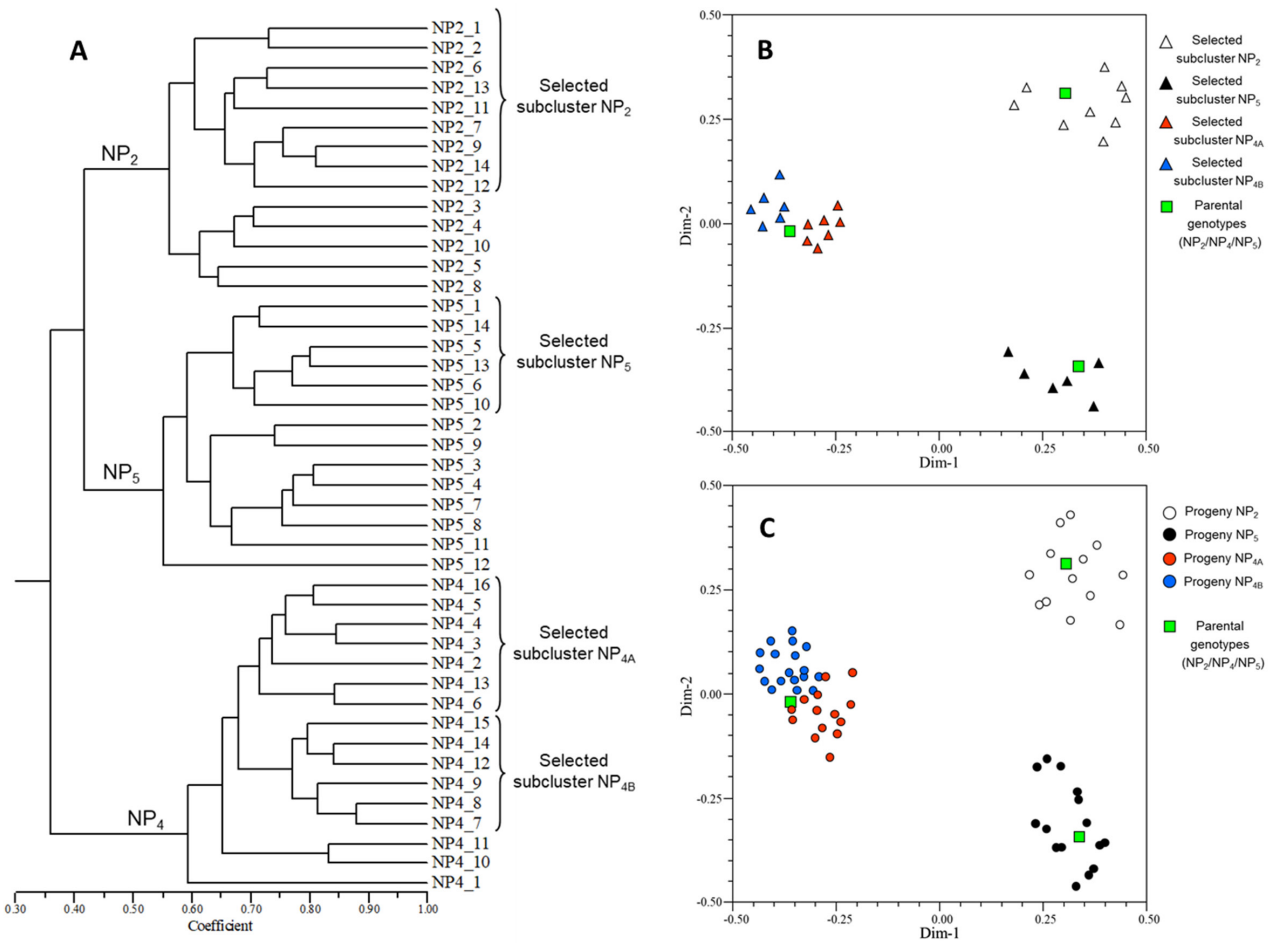

Figure 3. Phylogenetic analysis based on AFLP fingerprints of the NP progenies obtained following selfing of the progenitor plants; retained individuals are indicated by curly brackets (A)**.** Principal coordinate analysis based on AFLP fingerprints of NP_2, NP_4 and NP_5 progeny derived from (B) selfing of the progenitor plants (season two) and (C) within NP type sib-mating (season three).

Table 1. The yield and phenotype of the NP_2, NP_4 and NP_5 types and their progenies. In the first season, the plants were vegetatively propagated, in the second season, plants were raised from self-pollinated seed, and in the third season, plants were raised from seed produced by sib-mating within each NP group. Data for capitula weight per plant are not reported for season two, as the capitula were used for seed production. Different letters shown within a given column indicate a significant difference between means, according to Fisher's LSD (P≤0.05)

Genotype	Season	Number of capitula/plant	Average weight of capitula (g)	Plant height (cm)	Length main stem (cm)	Diameter main stem (mm)	Lenght leaves (cm)	Width leaves (cm)
ALL	1st	17.5 a	125 a	100.6 b	34.0 b	27.1 a	129.9 b	68.7 b
	2nd	17.1 a	-	106.2 a	35.4 a	27.2 a	136.5 a	72.3 a
	3rd	16.6 a	130 a	103.9 ab	32.3 c	25.4 b	126.9 b	66.6 c
NP_2	1st	17.7 **+/-** 0.67	131 **+/-** 5.1	88.30 **+/-** 1.27	29.67 **+/-** 1.20	28.53 **+/-** 0.67	142.47 **+/-** 2.24	78.40 **+/-** 1.14
	2nd	17.3 **+/-** 0.70	-	96.40 +/- 0.61	32.10 +/- 1.10	28.67 +/- 0.23	148.90 +/- 0.75	81.47 +/- 0.96
	3rd	16.3 **+/-** 0.35	136 **+/-** 1.6	98.18 +/- 0.23	28.85 +/- 0.68	26.81 +/- 0.25	132.11 +/- 0.53	75.21 +/- 0.53
	Mean NP2	17.1 b	134 b	94.3 b	30.2 c	28.0 a	141.2 a	78.3 a
NP_4	1st	15.0 **+/-** 0.58	140 **+/-** 11.4	109.30 +/- 2.04	31.67 +/- 0.88	27.53 +/- 0.34	127.63 +/- 0.72	65.37 +/- 1.02
	2nd	14.4 **+/-** 0.43	-	112.67 +/- 1.98	32.60 +/- 0.76	28.33 +/- 0.38	136.33 +/- 1.21	69.47 +/- 1.11
	3rd	14.7 **+/-** 0.35	140 **+/-** 3.6	103.27 +/- 0.62	30.27 +/- 0.08	25.64 +/- 0.30	130.0 +/- 0.33	62.35 +/- 0.21
	Mean NP4	14.7 c	140.0 a	108.4 a	31.5 b	27.2 b	131.3 b	65.7 b
NP_5	1st	20.0 **+/-** 1.35	105 **+/-** 11.0	104.19 +/- 2.29	40.73 +/- 0.91	25.33 +/- 0.43	119.53 +/- 1.48	65.37 +/- 0.86
	2nd	19.7 **+/-** 0.77	-	109.67 +/- 3.49	41.63 +/- 0.81	24.67 +/- 0.41	124.43 +/- 2.46	66.00 +/- 1.31
	3rd	18.7 **+/-** 0.73	113 **+/-** 6.3	110.18 +/- 2.35	37.93 +/- 0.49	23.69 +/- 0.35	118.52 +/- 1.18	62.31 +/- 0.71
	Mean NP5	19.4 a	110.0 c	108.0 a	40.1 a	24.6 c	120.8 c	63.6 c
LSD (P≤ 0.05) GxA		NS	NS	2.4	NS	NS	1.7	NS

NP_2, from about 23% to 20% in NP_4 and from about 24% to 21% in NP_5, and drove genome-wide homozygosity up to 79-80%. This was sufficient to depress seed set to 22% of the original level in NP4, to 46% for NP2 but just to 74% in NP5.

Although we confirmed that the increase of the homozygosity level causes a substantial penalty on reproductive yield (i.e. achenes production), the effect on seed setting was less marked in the NP5 than in NP2 and NP4, even though the former was the most homozygous. This means that the inbreeding depression other than associated to the homozygosity of the parental genotypes, is also genotype-specific as previously reported by Foury and Martin (1973) and Cravero et al. (2002), and its effect has to be assessed in field.

Differently, the homozygosity level of 85-90% induced by a further enforced sib-mating step had a very severe effect on seed setting in all the progenies (NP_2: 1.0%, NP_4: 2.1%, NP_5: 0.2%), sufficient to make it uneconomic to produce commercial quantities seed.

Our results confirm what previously reported on the effects of inbreeding depression in globe artichoke, which was found to be more marked after the second selfing generation and mainly affecting fertility and vitality of seeds other than capitulum traits (Basnizki and Zohary 1994).

Phenotypic variation

The performance of the three NP types over the three seasons is summarized in Tables 1 and 2. Capitulum number (averaged across the three seasons) was greater for NP_5 (19.4) than for either NP_2 (17.1) or NP_4 (14.7), and it did not significantly varied over the three seasons (Table 1). The heaviest capitula were produced by NP_4 (140 g), followed by those developed by NP_2 (134 g) and NP_5 (110 g) and also the average capitula weight did not significantly varied over the three seasons. No significant differenced were detected between NP_{4A} and NP_{4B} subcluster in the third season. Compared to those of the other two types, NP_2 plants were generally shorter, and formed a shorter main floral stem, which was larger in diameter. NP_4 and NP_5 plants were comparable in height, while the latter developed a longer, but narrower main floral stem. NP_2 plants produced the longest and widest leaves, and NP_5 plants the shortest and narrowest. In general, while both economic yield and morphology fluctuated over the seasons, there was little evidence to suggest that inbreeding depression acted to reduce vegetative (as opposed to reproductive) performance.

Table 2. Variation in seed- and fertility-related traits of the NP_2, NP_4 and NP_5 types and their progenies. In the first season, the plants were vegetatively propagated, in the second season, plants were raised from self-pollinated seed, and in the third season, plants were raised from seed produced by sib-mating within each NP group. Different letters shown within a given column indicate a significant difference between means, according to Fisher's LSD (P≤0.05)

Genotype	Season	Seed weight (g plant^{-1})	N° of seeds plant^{-1}	Weight 1000 seeds (g)	Index of fruit-set (%)	N° of flowers plant^{-1}	Not viable pollen (%)
ALL	1st	84.9 a	1914 a	46.2 ab	38.7 a	4933 a	13.8 c
	2nd	50.8 b	982 b	49.6 a	18,9 b	5176 a	14.7 b
	3rd	1.6 c	17 c	43.2 b	0.5 c	3698 b	18.9 a
NP_2	1st	98.0 **+/-** 3.15	1892 +/- 104.27	52.6 +/- 1.81	41.2 +/- 0.01	4557 +/- 256.19	11.5 +/- 0.09
	2nd	45.0 +/- 4.14	874 +/- 118.07	52.3 +/- 2.37	17.4 +/- 0.02	4980 +/- 177.04	11.6 +/- 0.03
	3rd	0.8 +/- 0.39	18 +/- 8.06	45.5 +/- 1.45	0.4 +/- 0.00	4235 +/- 84.76	11.3 +/- 0.12
	Mean NP2	47.9 a	928 ab	50.2 a	19.7 b	4591 a	11.4 b
NP_4	1st	56.4 +/- 9.10	1504 +/- 261.24	42.5 +/- 0.78	31.8 +/- 0.02	4729 +/- 557.49	20.7 +/- 0.41
	2nd	14.4 +/- 1.45	328 +/- 22.42	43.3 +/- 1.69	6.2 +/- 0.01	5289 +/- 505.42	23.5 +/- 0.15
	3rd	3.7 +/- 2.96	31 +/- 10.71	39.0 +/- 1.28	1.0 +/- 0.00	3190 +/- 227.83	35.6 +/- 1.06
	Mean NP4	24.91 b	621 b	40.7 b	13.0 c	4403 a	25.3 a
NP_5	1st	100.4 +/- 22.40	2345 +/- 595.66	43.4 +/- 2.29	42.6 +/- 0.04	5514 +/- 810.16	9.2 +/- 0.12
	2nd	92.9 +/- 6.17	1744 +/- 155.99	53.2 +/- 2.02	33.2 +/- 0.08	5257 +/- 819.63	9.1 +/- 0.07
	3rd	0.2 +/- 0.08	4 +/- 1.92	47.0 +/- 0.64	0.1 +/- 1.00	3669 +/- 115.59	9.8 +/- 0.19
	Mean NP5	64.5 a	1364 a	48.1 a	25.3 a	4780 a	9.4 c
LSD (P≤ 0.05) GxA		12.7	NS	2.4	4.6	NS	0.5

The number of capitula produced per plant did not differ significantly between plants of a given type grown caged or in the field (data not shown). However, the number of achenes produced by caged plants, which benefited from enforced pollination by bumble bees, was around 30% higher (data not shown). The germination rates of NP genotypes were 86% (NP_5), 80% (NP_2) and 68% (NP_4) while the mean germination times were, respectively, 6.3, 6.7 and 5.7 days. The mean 1,000 seed weight ranged from 40.7 g (NP_4) to 50.2 g (NP_2) (Table 2). Achene set (and hence also seed yield) declined over the second and third seasons, falling to very low levels by the third season (from 1,892 to 18 seeds per plant for NP_2, from 1,504 to 31 for NP_4 and from 2,345 to just four for NP_5 (Table 2). On the other hand, there was little variation in 1,000 seed weight between generations. The number of flowers per plants produced by each of the three NP types did not differ significantly, but it suffered a decrease in the third season, amounting to some 33% in NP_4 and NP_5 and just above 7% in NP_2. Due to the fall in seed set, the FSI fell globally from 38.1 to 0.5 over the three seasons (Table 2). Pollen viability was highest in NP_5 (>90%).

A further interesting result we obtained is the evidence of the relative insensitivity of vegetative vigour to inbreeding, specifically in terms of the number and weight of capitula produced. The capitulum yield of the NP progenitors (and their derivatives) corresponded to a production level of 21-23 t ha^{-1}, which was about double the productivity of standard vegetatively propagated varieties and of the same order as that of commercial F_1 hybrids. Additional features of all three NP materials were that their harvest period lasted just a month and a half, their capitula were very compact, their bracts were free of anthocyanin and there was only a limited development of floral pappus on the receptacle (data not shown); together this ideotype is well suited to industrial processing.

CONCLUSIONS

On the whole our results highlight that after just one cycle of selfing of phenotypically selected plants, thanks to an AFLP-based selection, it was possible to identify in the progenies a set of genotypes which sib- mated in isolation cages and in presence of bumble-bees, produced seed lots originating high yielding and phenotypically uniform populations which meet the DUS (distinctivity, uniformity and stability) requirements of a new variety. The set of sib-mated genotypes best performing in terms of achene production (which in our case was NP_5) can be easily vegetatively propagated and this allow modulate the production of the seed in relation to the commercial needs. In the context of considering these materials as prospective varieties for release, it will of course be necessary to perform larger scale, multi-location trials over several seasons, both to validate their yield performance and confirm that they satisfy the required distinctness,

uniformity and stability criteria.

The selection approach described here could be readily adopted to convert current vegetatively propagated landraces into seed-propagated varieties. Such a conversion would simplify and reduce the cost of current cultivation practices, as well as reduce the losses caused by the presence of systemic pathogens. Since landraces are typically highly heterozygous, more than one cycle of selfing will probably be needed before phenotypic and marker assisted selection can be imposed. The model would be to bring the level of homozygosity up to around 80%, a level at which inbreeding depression, at least in some genotypes, is likely to be only mild on fruit setting without affectind the production of capitula. Note that the breeding of the first seed-propagated variety ('Green Globe') required a 20 year period of mass selection (Pecaut 1993), yet still is only 40% homozygous and that Many years were also required for the development of the seed propagated variety 'Talpiot', the first one introduced in cultivation in Europe (Basinzki and Zohary 1987).

The globe artichoke genome sequence has recently been released (Scaglione et al. 2016, Acquadro et al. 2017), releasing a wealth of sequence data exploitable for marker development. Here, reliance was placed on an established AFLP platform, which has been used extensively for the genetic analysis of globe artichoke (Acquadro et al. 2009, Mauro et al. 2012, Mauro et al. 2015). While now generally superseded by other DNA-based marker systems (particularly those targeting single base variants), AFLP technology still remains a convenient and informative platform for marker assisted breeding, particularly for small-scale programs which cannot afford the capital investment needed for assaying single nucleotide polymorphisms (Zhang et al. 2014), while the re-sequencing of many individuals in most situations is unnecessary and would inflate the costs.

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