Europ.J.Hort.Sci., **72** (6). S. 241–247, 2007, ISSN 1611-4426.

Anemone coronaria Breeding: Current Status and Perspectives

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Summary

The genus *Anemone* (*Ranunculaceae*) includes about 85 species. The Mediterranean basin is the centre of differentiation for many of the species that have contributed to presently cultivated varieties: *A. coronaria*, *A. hortensis*, *A. pavonina* and *A.* x *fulgens*. *A. coronaria* is, either in nature or under culture conditions, a winter-flowering species and the progenitor of the varieties currently grown for cut-flower production and garden ornament. Current cultivars cluster series of sub-cultivars which are characterised by quite uniform colour. Nevertheless other traits as flower production, precocity and flower quality show an evident genetic variation. *A. coronaria* may be considered a multifunctional species. It is expected that new cultivars will carry a set of distinctive traits that make them suitable for definite uses: cut flower production, garden or pot plants. In recent years, new tools that may support breeding activities were established: *in vitro* propagation of adult plants and seedlings, androgenetic plant production, characterization of populations and cultivars by molecular markers and detection of Single Nucleotide Polymorphism in cDNA. In consideration of the potentials of such tools, two major strategies are proposed for breeding new cultivars: A) crosses between selected heterozygous parents and B) crosses between inbreed homozygous or androgenetic parents in order to obtain true F_1 hybrids.

Key words. AFLP – anther culture – F_1 hybrids – molecular markers – *Ranunculaceae* – tissue culture

Botanical Features

The genus *Anemone* includes about 85 species (Bailey 1958), most of which are native to the temperate and mountainous regions of the Northern Hemisphere. The species are equally distributed across Europe, North America and Asia, but only few are endemic to South America or Southern Africa. The name *Anemone* is thought to derive from the Ancient Greek word *anemos* (meaning wind), an allusion to the frailness and lightness of the flowers, and was used already by Theocritus. Plant habit is typically herbaceous, with a perennial underground organ (tuber, rhizome or corm); leaves with long petioles and one to many erect flower stalks, which in their upper part carry a bract involucre near or remote from the flower. Five or more petaloid sepals, numerous free stamens and one ovulated carpel characterize the flower. Several *Anemone* species are cultivated for ornamental use and can be grown in a wide range of environmental conditions.

The Mediterranean basin is the centre of diversity for many of the species used to derive cultivated varieties; these include *A. coronaria*, (poppy anemone), *A. hortensis*, *A. pavonina* and *A.* x *fulgens* (Meynet 1993). Wild populations of *A. coronaria* are frequently found throughout the Eastern Mediterranean littoral, from Greece, through Southern Turkey and Syria, to Israel. Sporadic populations extend eastwards towards Northern Iraq and westwards along the Mediterranean shores of Italy, Southern France and Northern Africa (Horovitz et al. 1975). *A. coronaria* is a winter-flowering species, both in its natural habitat and when cultivated (Yonash et al. 2004) and is the progenitor of the majority of varieties currently grown for cut-flower production and garden plants.

A. hortensis, *A. pavonina* and *A.* x *fulgens* (the interspecific hybrid of *A. hortensis* and *A. pavonina*) are also popular as garden plants.

A. coronaria is mostly a cross-pollinated species. Its stigma is receptive ten days before the pollen is shed. Self-pollination is possible, but if enforced, generates inbreeding depression (Horovitz et al. 1975). The bees, flies and beetles attracted to the flowers to feed or collect pollen are capable of distributing pollen over long distances, thereby maintaining and enhancing the genetic variation of populations. Although the pollen exhibits many of the features typical for a wind pollinated plant (it is dry, stores carbohydrates, and has a diameter of less than 40 nm and an exine which lacks any ornate sculpture) the majority of the pollen is deposited within 1.5 m of its source (Horovitz 1991). Each flower produces 200–300 seeds, covered with a woolly or glabrous coat.

Cultivation

A. coronaria is grown over the winter for cut flower production and in late winter to spring as a pot and garden plant. Seeds harvested in spring are usually germinated in late summer or early autumn. Seedlings transplanted in pots, start flowering the following spring. Tubers are

frequently used by growers in order to shorten the time from planting to harvest. These can be derived from seed within one growth cycle in the nursery. Intact tubers, rather than fragmented materials, are usually preferred for phytosanitary reasons. To bring forward the flowering by an additional four weeks, tubers can be vernalised before planting (OHKAWA 1987). The process consists of four major steps: soaking in water for 24–48 h; disinfection with fungicide; four to five week storage at ~5 °C and planting of sprouting tubers in the field or greenhouse. In the Mediterranean temperate coastal area forced tubers, planted in the open field in the second half of August (week 33), start to flower at the beginning of October (week 39) and continue to do so during winter and spring, providing a source of cut flowers for a period of some six to seven months. In cooler regions, greenhouse protection and supplementary warming is required. Under controlled conditions, the highest number and the best quality of *A. coronaria* flowers is produced by a regime of 5–10 °C at night and 14–18 °C during the day (OHKAWA 1987).

Cultivars

The history of *Anemone* cultivation has been reviewed by NICOLINI (1969). Pliny recorded the presence of both wild and cultivated forms of *Anemone* during the Roman era. Dioscorides reinforced Pliny's opinion, confirming that many *Anemone* types were under cultivation during his time. At the end of the Middle Ages, Ferrari listed several *Anemone* varieties in his "Florum cultura" (1631). *A. coronaria* must have been introduced to England before 1596, as it was described and faithfully portrayed in the floricultural volume "Johnson's Gerarde" and was very popular during the reign of Queen Elizabeth I (1558–1603). By 1629, Parkinson was listing 60 species and varieties of *Anemone*. By the beginning of the 18th century, French and Italian breeders had made substantial contributions to improving the range of colours available. Modern cultivars boast very large flowers (of diameter 8–10 cm), with a wide range of both bright and pastel colours; dual coloured types have also been bred. Anther colour is usually black, but is pale green in white flowering types. Stems reach a height of 40–50 cm and each plant produces 13–15 harvestable blooms.

A. coronaria cultivars are classified into the two major groups: 'De Caen' and 'St. Brigid'. The former represents a collective name for single flowered cultivars, such as 'Hollandia' (purple), 'Mr. Fokker' (blue), 'Sylphide' (mauve), 'The Bride' (white), and 'His Excellency' (scarlet with a white eye). The 'St. Brigid' group includes cultivars with semi-double and double flowers, such as 'The Admiral' (dark pink), 'The Governor' (carmine red), 'Lord Lieutenant' (blue-violet), and 'Mount Everest' (white). Two minor groups are also recognised: 'Rissoana', consisting of very rustic types which flower precociously (in November) and 'Grassensis', typified by their large double flowers, blooming in spring. Most modern cultivars have been derived either by selection from within old 'De Caen' types, or from crosses between 'De Caen' cultivars and selections from wild populations. 'Wicabri', 'Mona Lisa', 'Cristina', 'Jerusalem', 'Tetranemone', 'Mistral' and 'San Piran' are cultivated throughout Europe, the USA and Israel and include a number of sub-cultivars distinguished by their flower colour. Cultivars bred for cut flower production are characterized by prostrate leaves and by long stemmed flowers. For pot plant production, these traits need to be adjusted by the appropriate supply of growth retardants.

Anemone Breeding

Breeding aims

A. coronaria is a multifunctional species, though recent cultivars have been largely selected for cut flower production. Besides more general breeding aims such as product uniformity, resistance to diseases and to high temperature, flowering under long photoperiods, flower colour and vase life improvement, new cultivars need to carry a set of distinctive traits which make them suitable for particular uses. Thus cut flower types need to flower early, have robust stems and large multi-sepaled flowers; while garden and pot plant types need to have a compact growth habit, erect leaves with a short petiole, profuse and coordinated flowering.

Current status

In choosing appropriate genetic improvement strategies, breeders have to take the following features of *A. coronaria* into account: allogamy, protogyny, inbreeding depression, slow generation turnover (one per year), sufficient seed production, technical difficulties associated with vegetative cloning needed for phytosanitary reasons, low genetic variation for economically relevant traits (e.g. resistance to diseases, specific colours). The limited financial return from breeding, because of the relatively low economic importance of the species, has also played a significant role. However, renewed interest in *A. coronaria* is now expected, on the basis of its relatively low energy requirement.

Current cultivars cluster into a series of sub-cultivars, which are defined on the basis of flower colour; within each cluster, other traits, such as flower yield, precocity and flower quality can be genetically variable. This non-uniformity reflects the dual aims of breeding, which are first to maintain a high level of vigour and second to provide a uniform product. Sub-cultivars seed lots are prepared by deliberate hand crossing selected plants of distantly related families, which are maintained by sib crosses (Fig. 2a). Thus individuals within each family are typically heterogeneous and partially heterozygous, so that progeny are inevitably non-uniform. In a final step, a commercial seed lot of each sub-cultivar is generated by the bulking of several crosses. Sub-cultivars are therefore plant populations and their genetic composition can vary through the years as a consequence of seed lot assembling with material of diverse origin.

A quite different breeding strategy to breed new varieties, used mainly in France, exploits synthetic autopolyploidisation. The cultivar 'Tetranemone', bred from 'Wicabri' using this technology (MEYNET 1993), is characterised by greater flower stalk length and width, sepal thickness and flower size. Its flowering time was delayed in autumn (GOUJON et al. 1979). Recently, JACOB et al. (1997) induced tetraploids in 'Mona Lisa' and compared 'Tetranemone', tetraploid 'Mona Lisa' and the 'Tetranem-

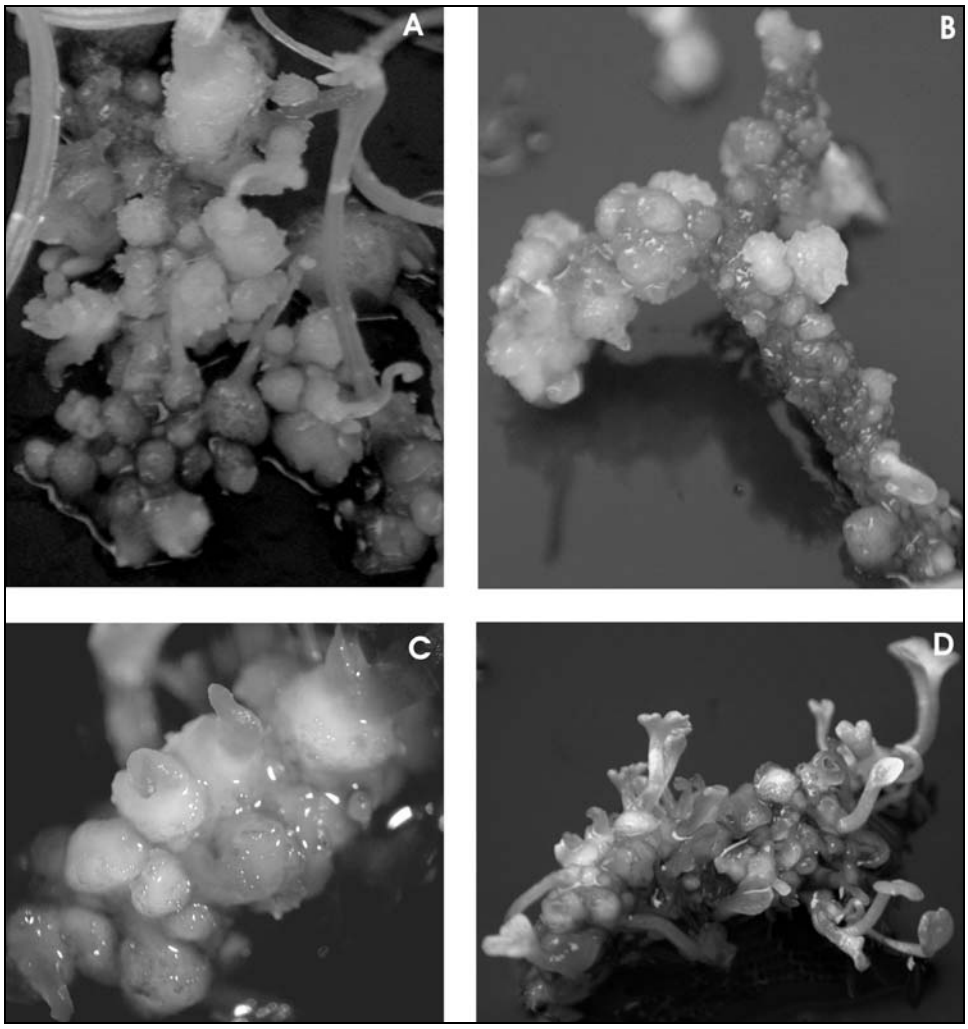

Fig. 1. Regeneration from androgenetic tissue of *A. coronaria*: A: Recurrent embryogenesis; B and D: Early and late phase of plantlet regeneration from roots; C: Detail of early events of regeneration. All plant tissues were grown on medium used to rescue anther-culture derived embryos (LAURA et al. 2006b).

one' x tetraploid 'Mona Lisa' hybrid with their originating diploid cultivars. The hybrid maintained the tetraploid advantage, was as early flowering as 'Mona Lisa' and produced a comparable number of stems. Triploid genotypes (GOUJON et al. 1979) both combined the productivity of the diploid progenitor cultivars and some of the advantageous traits of the tetraploids. The introgression of new genetic variation (e.g. yellow flower colour, erect leaves) was attempted by crossing *A. coronaria* with some of its related species. However, interspecific sterility was found to hinder the transfer of useful genes (MAIA and VENARD 1974 cited in: GOUJON et al. 1979). Similarly hybrids between 'Tetranemone' and *A.* x *fulgens* were sterile, though both parents are tetraploid, sharing the same somatic chromosome number (2n=4x=32) (Allavena, unpublished results).

New breeding tools

In recent years, a number of techniques to support the conventional breeding of *A. coronaria* have been developed. These include the *in vitro* propagation of adult plants and seedlings, the generation of androgenetic plants, the *in vitro* regeneration from somatic tissue, the genetic characterization of populations and cultivars using molecular markers and the discovery of single nucleotide polymorphisms (SNPs) in expressed sequences.

RUFFONI et al. (2005) established clones of selected plants *in vitro* from the portion of the floral stalk, overlapping the bracts. This approach overcame the problem of contamination encountered with tuber-derived explants. Up to 47 % of the genotypes tested generated bud primordial when cultured on a medium containing zeatin and a histological analysis revealed that *de novo* meristematic patches were formed, which went on to develop into adventitious buds. Since shoots and new flowers do arise sporadically at the base of the floral bracts in nature, the ability to generate shoots from pre-existing meristems must be also possible. The *in vitro* formed shoots were micropropagated and successfully acclimatized after cultivation in presence of Indole-3-butyric-acid to root and enlarge the basal tuber to a threshold weight of 0,5 g. The mature plants developed from these explants were uniform and showed no outward sign of somaclonal variation. Clones were also derivable from seed explants.

JOHANSSON and ERIKSSON (1977) established successfully anther cultures in certain wild *Anemone* species. Based on this protocol, LAURA et al. (2006b) regenerated somatic embryos and plantlets from anthers of *A. coronaria* elite cultivars: 'Cristina' (white, blue, pink, red, fuchsia, purple), 'Mona Lisa' (white, blue, red, purple, bicolour red and bicolour blue), 'Tetranemone' (white, blue), 'Wicabri' (blue, red, fuchsia, white, bicolour red) and 'Tetra

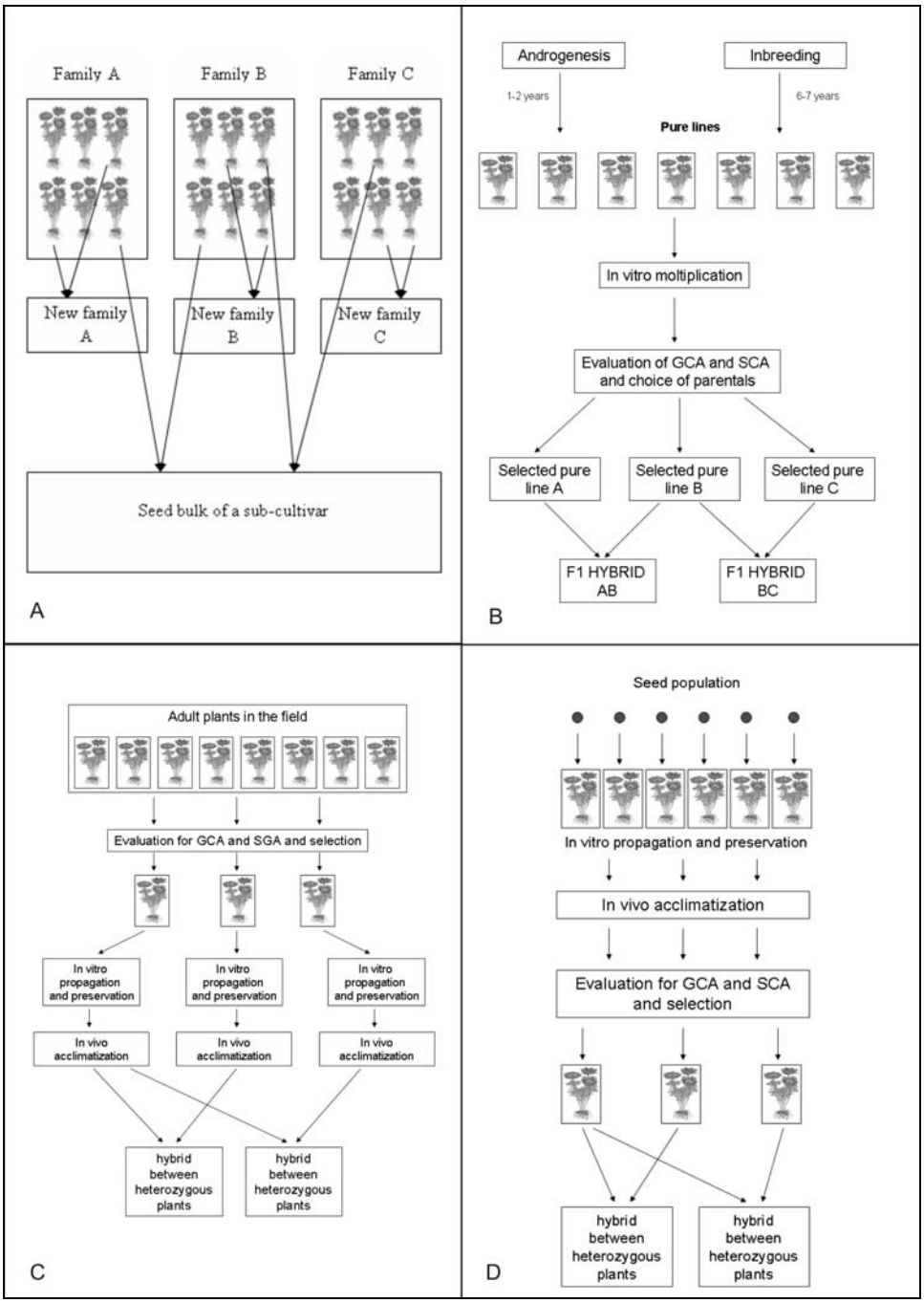

Fig. 2. Methods for seed production in *A. coronaria*: A: Conventional method (seed bulk of a given sub-cultivar is a mixture of crosses between plants of distantly related families); B) F_1 hybrid seed production (crosses between pure lines); C and D: Crosses between heterozygous plants to obtain the equivalent of a four way hybrid (original plant can be identified in the field or germinated *in vitro*). In schemes B, C and D plants were evaluated for their combinatory aptitude and propagated *in vitro* through the years.

Elite' (white). Up to 16.9 regenerants per 100 cultured anthers were obtained. The androgenetic origin of regenerated plants was ascertained by both cytological and molecular tests. Root apex chromosome counting showed that 2 of 19 regenerants were haploids and that 11 regenerants had either a 2x karyotype, or were x and 2x mixoploids. Two plants had 2x and 4x cells. RAPD-based DNA fingerprinting showed that all the regenerants tested differed genetically from their anther donor, confirming their androgenetic origin. Visual observation and molecular marker analysis proved that anther somatic tissue did participate neither in embryo formation nor in plant regeneration. Androgenetic plants were acclimatized in the greenhouse and produced flowers. A high regeneration capacity in term of recurrent somatic embryogenesis or adventitious bud formation from androgenetic embryos or from roots of regenerated androgenetic plants respectively was observed on medium used to rescue anther-culture derived embryos (Fig. 1; Laura unpublished; LAURA et al. 2006b). The high regeneration ability is thought to either have resulted from selection during the androgenetic process (genetic basis), and/or reflect an epigenetic effect associated with the juvenile state of explants.

A. coronaria has a 1C genome size of 9.08 x 10^9 bp and a singular to repetitive DNA ratio of 0.887 (WENZEL and HEMLEBEN 1982). The 1C DNA content of 8.45 pg, estimated by ROTHFELS et al. (1966), is roughly of the same range.

Due to the large genome size, the characterization of populations and cultivars by AFLP (Amplified Fragment Length Polymorphism) molecular markers was accomplished by modifying the Vos et al. (1995) procedure: 1) digestion with a single restriction enzyme (Eco RI) and running the gel for 5 hours (Nissim et al. 2004; Fang and Paz 2005); 2) DNA digestion with SbfI in place of Eco RI (Laura et al. 2006a). Nissim et al. (2004) characterized six wild populations growing in Israel and a commercial cultivar and observed a wide variation (80 %) within wild populations and much lower genetic variation in 'Mona Lisa'. One population (Dorot) and 'Mona Lisa' were found to have the largest genetic distance from the other wild populations and between themselves. Laura et al. (2006a) analysed four plants within each 24 sub-cultivars belonging to 'Cristina', 'Mona Lisa', 'Tetraelite', 'Wicabri' and 'Mistral'. The hierarchical analysis of variance demonstrated a high degree of differentiation within sub cultivars (approx. 42 %; P<0.001) and comparable levels of differentiation among sub-cultivars within cultivars (approx. 27 %; P<0.001) and among cultivars (approx. 30 %; P<0.001). The extensive genetic variation within sub-cultivars was presumed to be a consequence of: A) breeders' efforts to avoid inbreeding depression by crossing individuals from distinct families within each sub-cultivar and B) blending seeds of several crosses. The extent of variation at the molecular level reflects the variation observed at the whole plant level. The UPGMA dendrogram clustered the cultivars into three groups: A) including 'Cristina'; B) including 'Mona Lisa' and 'Mistral' and C) including 'Tetraelite' and 'Wicabri'. These relationships are consistent with the known origin of the cultivars. 'Mistral' derives from 'Mona Lisa' (Biancheri personal communication) and 'Tetraelite' was derived from 'Wicabri' by polyploidisation (Meynet 1993). Shamay et al. (2005) discovered Single Nucleotyde Polymorphism in c-DNA (c-SNP) clones, comparing 'Mona Lisa' with wild populations of *A. coronaria*. Phylogenetic analysis of six wild populations and one commercial cultivar revealed high polymorphism within and low polymorphism between wild populations, reflecting previous finding using AFLP (Nissim et al. 2004). The commercial cultivars prove to be quite homogeneous and genetically different from the wild populations.

Breeding strategies

Increasing variation. Discouraging outcomes of interspecific hybridisation have hampered the introgression of new traits into *A. coronaria*. Recent micropropagation and androgenesis protocols (Ruffoni et al. 2005; Laura et al. 2006b) now may allow for the straightforward rescue of hybrid embryos, the generation of androgenetic plants from infertile hybrids, the reduction of the chromosome number of tetraploid individuals and the creation of fertile tetraploids, cumulatively opening a number of novel perspectives for interspecific hybridisation.

Embryo rescue is among the longest established and most successful of *in vitro* procedures and has been widely used for the production of interspecific and intergeneric hybrid plants where normal hybrid endosperm development fails. The tissue culture medium acts as an endosperm substitute for the hybrid embryo, allowing it to complete its development. The dissected embryo is the most widely used explant material for this procedure, but whole ovules or ovaries have also been cultured where the embryo is too small to manipulate without risk of damage. Embryo culture must be initiated before embryo abortion, but the rate of success improves as the embryo matures. Young embryos require a complex medium with high sucrose concentrations, while more mature embryos can usually develop on a simpler medium with low levels of sucrose. The recent definition of the basal medium requirement for *A. coronaria* is the first step towards routine embryo rescue.

Transfer of the foliage habit of *A.* x *fulgens* (erect leaves with short petiole) into *A. coronaria* would facilitate the breeding of cultivars suitable for pot plant culture. At the same time *A.* x *fulgens* is monomorphic with respect to flower colour (red only), and could be greatly improved by the introgression of other colours from *A. coronaria.*

Interspecific hybrids between tetraploid *A. coronaria* and *A.* x *fulgens* are possible, but sterility largely blocks their utilisation. Where hybrid plants do yield viable pollen, androgenesis can be attempted (Laura et al. 2006b) either to reduce chromosome number of the hybrid or to isolate individuals carrying a recombinant chromosome set (Kopecky et al. 2005). The genome of each androgenetic plant is unique, as it reflects the outcome of a single meiotic event in the parent plant. Thus a population of androgenetic plants, derived from a single hybrid plant, can be expected to include a large number of alternative allelic combinations. Furthermore, there is evidence that certain allele recombinations are expressed in androgenetic plants that otherwise remain "hidden" in the parent plant or in other hybrid derivatives (Zwierzykowsky et al. 1999). This "hidden variation" may prove to be a valuable resource for future plant improvement. Furthermore, the androgenesis route may avoid any problems related to poor male transmission that can arise during conventional breeding programmes due to pollen competition or to pollen-stigma interactions. This phenomenon, which can lead to the loss of certain allele combinations, is less likely to be an issue in androgenesis.

There is some evidence that androgenesis generates variation spontaneously in ploidy and so allows for the recovery of haploid, doubled haploid, triploid and tetraploid plants (Laura et al. 2006).

Aneuploids (chromosomes euploid complement with extra or missing chromosomes) can be exploited to investigate genic imbalance and chromosome pairing behaviour. It has been difficult to produce aneuploids by conventional breeding, but haploid plants carrying an extra chromosome (x = n+1) have been noted among the products of anther culture in rice (Wang and Iwata 1991). Aneuploid series have been obtained in a number of species (e.g. datura and wheat), by selection among the progeny of triploid x diploid or x tetraploid hybrids (Ezura et al. 1993; Parks et al. 1999) or among those from wide crosses (such *Lolium* sp. x *Festuca* sp.; Kopecky et al. 2005). Many such aneuploids can be used to assign specific genes or DNA markers to individual chromosomes. Since triploid *A. coronaria* has been successfully synthesised from tetraploid x diploid crosses (Goujon et al. 1979) and some interspecific hybrids are available, it is likely that aneuploids could be generated in this species for genetic studies and breeding. A possible means of achieving aneuploidy takes advantage of the partial male fertility of triploid hybrids derived from some tetraploid x diploid crosses (Jacob et al. 2006). When

the pollen from the triploid plants is used to fertilise a diploid, progeny with a variable chromosome number are produced. This so-called "interploid cross" has been used to improve flower colour in *A. coronaria,* and has been proposed as a general method for creating novel genetic variability.

Mutation breeding has been largely neglected in *Anemone*, possibly because of the large size of the progenies that need to be screened to identify recessive mutations. The use of anther culture derived haploids, combined with *in vitro* mutagenesis, may speed up the recognition of induced mutants, since mutations are not masked at the haploid level. Since mutagenesis frequently abolishes function, it may be particularly suitable to modify aesthetic characters such as flower colour, and plant architecture. WANG and LI (2006) have reviewed the inheritance of shoot branching, plant height and inflorescence form in a range of plant species. In *A. coronaria*, some potential targets for mutagenesis are the gibberellin-insensitive (*GAI*) and brassinosteroid (*BR*) biosynthetic or signaling genes, since these are all major determinants of plant height. In *Dianthus* and *Callistephus*, disruption of the chalcone isomerase, dihydroflavonol 4-reductase or flavanone 3-hydroxylase gene leads to the accumulation of yellow pigment (FORKMANN 1989; FORKMANN and MARTENS 2001). Yellow sepal colour is lacking in *A. coronaria*, but the flavonoid pathway is present, given that cyan, red and blue pigments are all expressed. Thus mutagenesis may be a practical approach to create yellow flowers in *A. coronaria.*

Molecular markers

Molecular markers can complement breeding programmes in a number of ways. Chief among these is marker-assisted selection (MAS), an approach which effects selection for a desirable trait by means of selection for a linked marker. Linkage is established by genetic mapping, and DH populations are widely used for this purpose. Because of their homozygosity, DH lines are genetically stable and infinitely replicatable and are thus ideal material for the genetic analysis of quantitatively inherited traits, since repeated trials are needed to isolate the environmental component of the overall phenotypic variance (LU et al. 1996; CHEN et al. 1996). Markers for MAS include Restriction Fragment Length Polymorphisms (RFLP), Random Amplified Polymorphic DNA (RAPD), AFLP and SSR. All have been used in combination with DH-populations for QTL (quantitative trait locus) mapping in the major crop species. Thus, for example, RFLP markers linked to a number of disease resistance genes have been identified in barley (GRANER and WENZEL 1992; GRANER and BAUER 1993). Anther culturability appears to be quantitatively inherited, under the control of several nuclear-encoded genes (MIAH et al. 1985; QUIMIO et al. 1990). Whereas it was earlier only possible to determine whether there were any differences in anther culture response between cultivars, and whether traits such as callus induction and plant regeneration were heritable, the development of MAS now allows the pattern of inheritance to be defined. Mapping genes for anther culturability would simplify the selection of individuals with a good response to anther culture and thus in the establishment of the DH populations required for the mapping of economically relevant traits.

Selection methods

Keeping in mind the particular sexual biology of the species, the need to sustain a high level of heterosis in order to maximise plant yield and quality, the market requirement for uniformity of product and the opportunity offered by new breeding tools, two major strategies involving a departure from conventional breeding practice are presented here (Fig. 2). The first involves intercrossing selected heterozygous parents to obtain an equivalent of a four way hybrid and the second intercrossing homozygous inbreeds or androgenetic derivatives to obtain true F_1 hybrids. In both cases, accurate characterization of parental lines with respect to their general and specific combining ability will help to arrange the lines into heterotic groups. Additionally parental choice can be aided by the judicious use of AFLPs, which have allowed plant material to be assigned to cultivar or even sub-cultivar (LAURA et al. 2006). The breeding of true F_1 hybrids will provide a high level of product uniformity, but suffers the substantial time and/or cost associated with the process of producing pure lines and later by the poor fertility of the parental, DH or inbred lines. On the other hand, hybrids made between selected heterozygous parents could speed up the breeding cycle, at the expense of some loss of crop uniformity. A trade-off between uniformity and productivity could be found by the synthesis of three way hybrids, made by crossing a highly fertile partially heterozygous line as female to an androgenetic line as male. Whatever the approach adopted, the preservation and multiplication of parent materials by tissue culture is essential for sustained success. The variable ploidy among androgenetic plants may facilitate the selection of the tetraploid lines needed to create tetraploid and triploid varieties.

Genetic engineering

The major limitation to date to genetic engineering in *A. coronaria* has been the lack of a reliable protocol for plant regeneration from somatic tissue. Recent observations that certain androgenetic plants undergo recurrent somatic embryogenesis or adventitious bud formation and the availability of wide host ranges *Agrobacterium tumefaciens* strains, have however opened new perspectives for *A. coronaria* genetic engineering. Mono- or oligogenic characters, such as resistance to diseases, tolerance to high temperature, absence of vernalisation requirement, increased vase life and flower colour, not available in either *A. coronaria* or any of its interfertile species, could, in principle, be readily transferred by genetic engineering.

Concluding Remarks

A. coronaria, as for most of the ornamental crops, occupies only a small share of the market. The modest economic value of the species has prevented the influx of any substantial capital investment. However, due to its low temperature requirements, interest in the species is expected to increase, especially in the Mediterranean basin, where the winter conditions (high light intensity and warm temperatures) are favourable for its cultivation. Over the last three decades, *A. coronaria* breeding meth-

ods have remained largely unchanged. However, recent advances in the fields of micropropagation, androgenesis and molecular markers present a number of new opportunities.

The means to micropropagate selected plants is now well established, so can be employed to propagate and maintain the parental material required for seed production. Androgenetic plants have been derived from elite cultivars suitable for cut flower production. DH technology provides a rapid means to achieve homozygosity, thereby accelerating breeding programs beyond the at least the six years required to breed pure lines by conventional selfing. Shortening this period to just one year should convince seed companies to invest in F_1 hybrid breeding, which carries with it in addition the important guarantee of uniformity and improved quality of the product. Horizon technologies are androgenesis to facilitate wide hybridisation (although this is still to be fully evaluated), the induction of genetic variation via mutagenesis, the establishment of MAS and the creation of new traits by genetic engineering.

AFLP provides an effective means for the evaluation of genetic diversity, and the genotypic data it has generated appear to be fully consistent with what is known about the origin of the cultivars. They also are informative in assigning seed lots, rhizomes, cut flowers or pot plants to a particular cultivar or even sub-cultivar and represent a way forward for the application of MAS in *A. coronaria* breeding and protection of breeder rights.

The final goals of breeding in *Anemone* are for the improved quality of the product, increase in genetic variation and the creation of cultivars adapted for specific uses, in particular cut flowers, garden and pot plants.

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Received September 26, 2006 / Accepted April 19, 2007

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