Who's still breeding flowers?

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To be clear
start with the conclusion.
w. shakespeare

POSITION OF THE PROBLEM

The flower sellers' customers, the only qualified to judge the value of the plant, are chiefly in search of beauty, and flowers, as subject of inspiration, cannot be less than perfect.

The beauty in flowers means perfection and for applied researchers, faultless quality, result in a more pragmatic goal.

However in the living matter beauty fades quickly, so durability is the second main feature essential to gaining competitive market value.

In this time (years 2007) the beauty and the durability are not enough;

incidentally growers but also customers whish plants producing a continuous flowering: in short a "flower machine".

The international flowers market show worries caused by overproduction and money shortage to spend for flowers

Modern people whish plants enjoying the everyday life, making more comfortable stress relievers work places, air purifiers environment, symbols of feelings, friendship, in short for the well being and beautification of life. Definitely multi purposes plants, diversify in using, are the future strategy to face the market answering this tomorrow needs (ex. adorn diner table, etc).

The western European customer, is continuously questing for novelties, new varieties, setting in this way a basic conflict in the ornamental production research conservative strategies as for instance those based on the F1 hybrid .

In fact a continuous fast flow of plants varieties with new features new colour, design, pattern and shape, always diverse from the pre existing types may set a questionable matter about the high costs of the biotech research and the transgene technologies, in particular.

Many ornamental species should be reconsidered under this point of view, taking in account of course the large natural genetic variability existing in these genomes still waiting to be explored and exploited with conventional methods, less expensive (low energy) and plain, not requesting sophisticated equipment and high specific competences and last but not least the issue of genes patents, banning the free utilization on genome, and subtracting the free access to the gene plant resources.

The basic goal, for ornamentals is to implement a continuous flows of varieties assortment only possible having a large germplasm core collection, representing the genetic variability actual and potential of the specie plant genome. So the basic aspect for the breeding work, is to maintain, preserve, improve and exploit a effective sexual reproductive system inside of a most large differentiated germplasm.

Growing collections of eligible germplams, eventually in different locations allow to exploit interaction from genotype / environment, a phenomena well know by breeding firms that deliver different catalogue accordingly the different enviromental conditions (roses, gerbera, alstroemeria are just some example this differential reaction). In this task private collectors , gardens amateurs, nurseries, can play a dramatic role supplying the plant material to the breeders. The English network of appointed nurseries, specialised for one or few species answer this needs in a very cost-effective system.

Parent performance and their evaluation in general and specific combining ability is the first step in the breeding work. This design, if well organised permit to develop long planning and future strategies making possible to fit promptly the new unpredictable trend of the market. This plant material should be considered a open source germplasm, where no Patent (genes) act to limit the access to the gene pool.

Furthermore, there is no needs of labs and/or special expensive competence to manage this basic germplasm.

Main constraints for the breeder may came from the lacking of fertility in his germplasm collection. Some time, sterility factors may bypassed by the stilum fecondations, as demonstrated effectively in breeding lilium intercrossing program in Netherlands.

But usually recalcitrant or poor effective mother plants should be promptly withdrawn from the crosses design to avoid to carry inside bad genes for sterility. The presence of sterility factors (gene) of sporophytic or gametophytic may hamper dramatically the success of selection strategies.

Sterility some time depend to the ploidy level of the genotype that produce unbalanced and/or steril gametes. In this case the application of aploids protocols /strategies can allow the recovering fertile genotypes endowed of special useful genes.

Allogamous natural populations are often affected by lethal and sub-lethal genes due to the eterozygote or polyploid status that hamper dramatically the inbreeding progress. Indeed in some species as Gerbera, Roses, the auto-fecundation are impracticable cause the serious shortage of the offspring cause by genetic load.

Size of populations of core collection should be planned accordingly the amount of genetic variability of germplasm after a statistical estimation of genetic variability

HOW TO DEVELOP THE POTENTIAL GENETIC VARIABILITY:

Still today the interspecific, -in some way forcing the evolution trend- represent the most effective methods to enlarge genetic variability obtaining gene combination otherwise absent inside of the single species. Examples refer to the most important popular flowers: rose, carnation, liliums, chrisantemum, orchids, dahlia, hibiscus,gerbera, freesia, gladiolus, etc

By *in vitro* manipulation embryo of special interest can be rescued and developed obtaining plants with special combination genes.

The Pollen selection is another long term strategy just recently reconsidered also by international breeding firm. In fact, gene expressed in the pollen (Gametophyte) are round to 60-70% of gene expressed in the sporophyte (Plant). So we can imagine to apply a strong selection pressure on the million pollen grain and so pollinate with pollen screened for some factor. Just for example the pollination in the cool months foster naturally and selection of the most microterm gamete, and so year after year we will get microterm plants.

Pollen selection was applied in carnation to get genotype Fusarium resistant. The system really work effective but is under applied because it require an efficient reproduction system, often lacking in our species,

So, also in this case, it is important to build a population with high frequency of favourable alleles and free of genetic load.

The sampling of starting germplams issue, remain the central task to get before organise a selection design. In short, we must improve the genetic structure of population building a elite high frequency sound allele population through screening out worse genes and moving towards the high frequency the best parental combinations. This core collection should be continuously will be improved by new elite accessions.

Considering F1 hybrid design not strategic cause the continuous flow of varieties assortment requested from the market, for our allogame and very eterozygote plant material, an effective system is to organize a diallelic design to test de combining ability. The best combining parent selected will be crossed and the best progenies cloned (vivo or vitro) afterward tested.

FOLLOW UP

The implementation of a pool genotypes with a great genetic variability (actual and potential) will allow to exploit (to extract) the variability (new varieties) year after year, accordingly input of market (customer, seller, grower, dealer, carrier) that remain largely unpredictable.

It need to organise a gene stock plants “in vivo” materials bred on site (possibly in different environmental area) and continuously improved by accession of new genotype.

The Micropropagation may set the cost issue: in fact often the added value of the clone from in vitro can’t afford the cost of “in vitro” multiplication. Gladiolus hybrids is just one example.

Furthermore non all genotypes fit the micropropagation protocols: some time it needs to select stable genotype, free of transposon (mobile elements) that, cause the hormone application may provoke instability in "*in vitro"* propagate plant material.

For this reason, aiming self-rooting genotypes to lowing costs of lab/micropropagation is always a sound strategy.

Finally, the use of Molecular Markers for plant/parent assessment and parental lineage to manage the breeding program appear nowadays unavoidable

BIOLOGICAL/TECHNICAL FEATURES

An efficient reproductive system both sexual and asexual is the first step of the work.

Aim to get genotypes fit to accept intensive growing system and flower production (schedule cropping attitude is very important in ornamentals cause the calendar of market request)

The good reaction (development) after pruning is very important for the management of the plant size and flower flow.

Look for low energy (heat/cool/hand powers/chemicals, etc) varieties and consider the plant as "flower machine" (es. rose, gerbera, anthurium)"

COMMERCIAL FEATURES

The test for durability/conditioning/air travelling/quality / perfection/beauty/ size, fit for delivering.

Plan for new and creative purposes, sunny colour, not trapping light.

Consider the modern trends: people has not time and whish flower in bunches bought in supermarket "ready to get".

Look for the future trends, more than ornamental purpose: able to answer different use: beautification and enjoying life: ex. air purifier, medicinal, edible, cosmetic, insect repellent etc?

SOME HISTORY CASE:

1) Pollen selection in Carnation for resistance to *fusarium oxysporum* f. sp. dianthi.
Mercuri, T. Schiva, G. Baratta, G. Fenoglio and G. Burchi
Acta Horticulturae 307, 1992 Carnation Culture

ABSTRACT

Pollen grains of 8 commercial varieties of Carnation were cultured "*in vitro*" on a germination medium with increasing concentrations (0%, 7,5%, 15%, 30%) of axenic culture filtrate of *Fusarium oxysporum* f.sp. dianthi pathotypes II and IV. The pollen response to the filtrate was observed as germination percentage and tube growth. The effect of the culture filtrate was also studied on sporophyte (seedlings coming from different crossings). The results show that the screening of resistant cultivars through pollen assay appears feasible.

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2) Interspecific crosses on alstroemeria

A.F.C. Tombolato, G. Burchi, A. Mercuri, C. Bianchini & T. Schiva

Proc. of the XVIIth Eucarpia Symposium "Creating Genetic Variation in Ornamentals".Sanremo, 1 - 5 March, 1993.

ABSTRACT

Alstroemeria (Alstroemeriaceae family) is a species from South america which is cultivated for cut flower production and for pots. It shows a high market potentiality for the future because of its agronomical and commercial characteristics.
At the Aalsmeer market, in Holland, one of the biggest flower trade center in the world, Alstroemeria sales increased four times between 1979 and 1988, from 17,749,130 to 74,749,130 stalks sold per year (Martorell, 1992). In the last years Alstroemeria is among the first ten cut flower species in the market.
The main Alstroemeria cultivars, nowadays available for the growers, have been selected in Holland with conditions of low temperature, low light and high humidity. So, many of these cultivars are not suitable for tropical and sub-tropical environments.
An Alstroemeria breeding program was established in collaboration between the Instituto Agronômico at Campinas - SP (Brazil) and the Istituto Sperimentale per la Floricoltura of Sanremo (Italy), aiming to cross new Brazilian and Chilean species and commercial varieties, and to select new hybrids for the cultivation in subtropical climates from a pool of less known species (Tombolato *et al*., 1992).
A review of the genera Alstroemeria was published by Bayer (1987) in a work on the chilean group. In this paper 31 species and 19 subspecies native from Chili are described.
Therefore, the Brazilian species remain unknown and there is a big confusion in their nomenclature. Traub (1973) emphasized the importance, for the breeders, of a correct description of native species. Aker & Healy (1990) cited 29 different epithets for Brazilian species, but it is possible that there are synonyms and certainly there are new unlabelled species. It is impossible to affirm if they are more or less numerous than the Chilean ones. In this manner, the plants in the collections, specially the Brazilian ones, are maintained by their collecting numbers and the Brazilian species are mentioned in this article just by their numbers.
The main characteristics of the parental genotypes involved in the breeding program are:
- Brazilian species; vigourous and rustic plants, mostly evergreen with large leaves and non stop flowering all the year around; they are usually diploids (2n=16)
- Chilean species; plants present interesting flower shapes, usually with big size of the tepals and in almost all colours; they are also usually diploids
- Commercial varieties; they were mainly originated from crosses between Chilean species. Brazilian species were rarely involved in their origin. Plants show interesting agronomical characteristics, specially high productivity of very long and flowered stems. The plants can be di-, tri- or tetraploids, and also aneuploids (Tsuchiya *et al*., 1987).
Interspecific hybridization, specially between the Chilean and the Brazilian groups, presents many difficulties like crossings-barriers and embryo abortion which justify the employ of *in vitro* techniques (De Jeu *et al*., 1992).

Mutation is also reported to be applied for Alstroemeria improvement. Several tens of varieties in cultivation are solid induced X-rays mutants (Broetjes, 1981 and Malluszynski *et al*., 1992).
Cariotype analysis is very important for the breeding of Alstroemeria because of the different ploidy levels (Tsuchiya, 1987) and also to understand the plant origin in the case of old cultivars (Buitendijk, personal communication).
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3) Breeding of Alstroemeria through interspecific crosses and embryo-rescue
Gianluca Burchi, Antonio Mercuri, Tito Schiva
Proc. Of the 2nd Int. Symp. on Ornamental Palms and other Monocots from the Tropics.Eds. M. Caballero Ruano Acta Hort. 486,: ISHS 1999

ABSTRACT

At present, with more than 130 cultivars, *Alstroemeria* shows one of the best trends in the international market for cut flower production. Most of these cultivars have been selec-ted in the Netherlands, in cultural conditions of relatively low temperature and light, and high humidity. In the Italian environmental conditions, most of these genotypes show a poor flowering ability in the winter season (the most important for cut flower production): for these reasons a breeding program, utilising Brazilian and Chilean species, was established. The ultimate goal is to select new genotypes suitable for cultivation in the Mediterranean area and to obtain new colors and new architectures of inflorescences. The major constraint in this goal is the presence of genetic barriers that provoke embryo abortion in interspecific hybridization, in particular between the Chilean and the Brazilian genotypes. To overcome this obstacle, an "in vitro" protocol for embryo-rescue of recalcitrant crosses was developed. The results of this breeding activity, reported in this paper, show that it is possible, through "in vitro" embryo-rescue procedures, to recover interspecific hybrids from botanical species usually unable to produce progeny when crossed.

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4) Use of rapd analysis for genotype identification in Alstroemeria
L. De Benedetti, G. Burchi, A. Mercuri and T. Schiva
Proc. 19th International Symposium Improvement Ornamental Plants.Ed A. Cadic Acta Hort. 508, ISHS 2000

ABSTRACT

Alstroemeria is a monocot ornamental crop originating from South America, with two centres of diffusion in Chile (highlands of Andes) and in eastern Brazil (tropical zones). Alstroemeria cultivars

are being developed through interspecific hybridization (using in some cases embryo rescue techniques to overcome crossing barriers), selection of sports and/or polyploidization (Broertjes and Verboom, 1974; Bridgen *et al*., 1989).
The development of genetic markers for the characterization and identification of genotypes and early detection of interspecific hybrids may be very useful for breeding programmes and in protecting the rights of the breeders. In recent years, RAPD (Random Amplified Polymorphic DNA) markers have become widely used for genotype identification in plants including ornamentals (Wolff *et al*., 1993; Weising *et al*., 1994).
RAPD analysis was performed on Alstroemeria samples with the aim to discriminate, identify and characterize new genotypes obtained through a breeding programme started in 1991 in our Institute in collaboration with the Instituto Agronomico of Campinas (Brazil).

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5) Random amplified polymorphic DNA (RAPD) analysis for the verification of hybridity in interspecific crosses of Alstroemeria
L. De Benedetti, G. Burchi, A. Mercuri, N. Pecchioni, P. Faccioli and T. Schiva
Plant Breeding 119, 443-445 (2002)

ABSTRACT

Random amplified polymorphic DNA (RAPD) markers were used to verify interspecific hybridization in Alstroemeria. Five putative inter-specific hybrids and their parents were analysed by means of four prese-lected RAPD primers. The putative parentage was confirmed in four hybrids and was excluded in one that showed completely different RAPD patterns from its putative parents and a different phenotype. Our results demonstrated that this molecular technique is a powerful tool for verifying hybridity rapidly if the putative parents are given. This tool will allow screening of small immature seedlings for verifica-tion of hybridity and should improve the efficiency of breeding pro- grams.

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G. Burchi, A. Mercuri, C. Bianchini, R. Bregliano and T. Schiva
Proc. 19th International Symposium Improvement Ornamental Plants
Ed. A. Cadic Acta Hort. 508, ISHS 2000

ABSTRACT

Most of Alstroemeria species have their center of origin in Chile, Brazil, Peru,Argentina, Venezuela, Paraguay and Bolivia (Wilkins and Heins, 1976), in latitudes ranging from 23° to 55°, in semi-desertic tropical zones up to the highlands of Andes, over 3000 m altitude.
Breeding of Alstroemeria via hybridization and X-ray mutation of rhizomes was done in England, in the Netherlands and in the USA (Goemans, 1962; Broertjes and Verboom, 1974). The commercial cultivars are interspecific hybrids with unreported parentage between Andean Chilean species and most of them are sterile triploids from crosses of spontaneous tetraploids with diploids (Heins and Wilkins, 1979). These cultivars have been selected in cultural conditions of relatively high humidity and low temperature and light. In the Italian environment, these genotypes show a poor flowering ability in the winter season because of the lack of cool soil conditions, essential to induce flowering (Aker and Healy, 1990).
The Brazilian genotypes were rarely involved in breeding programs. They have vigorous plants, mostly evergreen with large leaves, non-stop flowering all around the year and they are well adapted for cultivation in subtropical and mediterranean conditions (Tombolato *et al*., 1993). In 1991, the Istituto Sperimentale per la Floricoltura of Sanremo (Italy) and the Instituto Agronomico of Campinas-SP (Brazil) planned a breeding program crossing wild Brazilian species with Chilean species and commercial varieties, with the goal to select new genotypes suitable for the Mediterranean area. In this work, the first results of this activity are discussed.

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7) Results of a Breeding Activity on *Limonium spp.*
G. Burchi, E. Mercatelli and M. Maletta A. Mercuri, C. Bianchini and T. Schiva
Proc. XXIInd Int. Eucarpia Symp. (Sect. Ornamentals) on Breeding for Beauty Eds. A. Mercuri and T. Schiva Acta Hort. 714, ISHS 2006

ABSTRACT

A breeding activity on Limonium was carried out in Sanremo and Pescia since 1998. Wild species and commercial varieties were utilized in an incomplete diallelic cross design with the aim to obtain new varieties suitable for cultivation in Mediterranean conditions with low energy requirements. A first group of selected progenies derived from crosses among *L. latifolium, L. gmelinii, L. caspia, L. bellidifolium, L. otolepis and L. serotinum*. The first inter- and intra-specific hybrids were evaluated since 2001 in different environments. They were cultivated with minimum tillage and low input of fertilisers and pesticides showing, in these conditions, productivity and commercial quality comparable or also higher than the commercial control cultivars. A second group derived from the cross *L. bonduelli* x *L. sinuatum* (Statice). *L. bonduelli* is a Mediterranean wild species that needs

to be improved in relation to productivity, flower colour and stem architecture. Selected hybrids were evaluated in comparison with commercial cultivars of Statice. They showed a significantly higher production, significantly shorter and harder stems than *L. bonduelli,* a number of branches per stem significantly higher than *L. sinuatum* and commercial varieties, and different colour combinations of calyx and corolla. A third group derived from selected progenies of *L. tataricum* obtained from free pollination. These genotypes were characterized by good production, high number of flowers per stem and very attractive architecture. The last group derived from crosses among *L. aureum, L. sinensis, L. tetragonum, L. fortunei* and commercial cultivars. The new hybrids showed the valuable agronomical and ornamental traits of the parental cultivars and the stress tolerance of the botanical species.

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8) Use of RAPD Markers for the Genetic Characterization of Limonium Species
S. Bruna, L. De Benedetti, A. Mercuri and T. Schiva
Proc 21st IS on Breeding ornamentals, Part II Eds: G. Forkmann & S. Michaelis
Acta Hort 651, ISHS 2004

ABSTRACT

The genus Limonium (fam. *Plumbaginaceae*) consists of about 300 species of mostly herbaceous perennials, some low shrubs, and annuals. Most botanical species are endemics in the Mediterranean region, but many species have their center of origin in Caucaso, Turkestan, Caspian Sea, Russia, Iran, China, South Africa. Limonium is grown in several regions of the world for use as a cut flower for both fresh and dry-flower arrangements.
In this work, RAPD analyses were used for the study of genetic relationships in Limonium. Thirteen wild species were tested with 10 primers. A total of 244 bands were scored and used for the analysis of genetic distances. The dendrogram obtained from cluster analysis showed high similarity among three species that some authors report as synonymous and that appeared very similar from our previous phenotypic observations (*L. caspia, L. bellidifolium* and *L. otolepis*). In order to clarify the genetic relationships, further analyses were carried out on several genotypes

belonging to the three species. The new dendrogram, obtained scoring 151 RAPD bands, show that the genotypes did not group in clear clusters. Analysis of molecular variance (AMOVA) confirmed this trend: the highest genetic variation resulted among genotypes and only 6,58 % of the total variation resulted among the species. These results suggest thata the species can be considered synonymous. The use of RAPD markers in our case was thus useful for clarifying the highly probable identity of the three Limonium species, in a plant genus that is notably of difficult interpretation.

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S. Bruna, L. Braglia, V. Casabianca, A. Mercuri, L. De Benedetti and T. Schiva

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ABSTRACT

The commercial varieties of *Hibiscus rosa sinensis* came from the interspecific crossing work carried out in the past, involving tropical and subtropical species: *H. arnottianus, H. kokio, H. denisonii* and other unknown plant sources. This explain the great variability expressed in morphological and agronomical features. The use of molecular markers in ornamental plant breeding is very considerable for several applications. We have used the AFLP (Amplified Fragment Length Polymorphisms) technique with the aim to characterize a collection of commercial varieties of *Hibiscus rosa sinensis* and to determine genetic similarities within them. 64 varieties of *Hibiscus rosa sinensis* and 4 genotypes of *Hibiscus syriacus*, included to assess the efficiency of AFLP method, were analyzed with 8 selected primer combinations. All cultivars were clearly distinguished by their molecular fingerprints; a total of 213 polymorphic bands were detected and used to construct a similarity matrix by means of Jaccard coefficient. The UPGMA (Unweighted Pair Group Method with Arithmetic Average) derived tree separated the cultivars into two main branches: the first one included all H. rosa sinensis samples, the second one properly grouped *H. syriacus* genotypes. Several sub-clusters were observed. These data confirmed the large genetic variability of our *Hibiscus rosa sinensis* germplasm collection. The AFLP markers applied in a larger genotype assortment could be useful to perform a more efficient breeding design.

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KEY WORDS
Carnation; Alstromeria; Limonium; Hibiscus Breeding