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Breeding Methodology Meets Sustainable Agriculture

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ABSTRACT

This review is about the contribution of plant breeding to sustainable agriculture. This contribution is based upon three main pillars: (i) The enrichment of the source material with landraces and old cultivars, accompanied by a breeding methodology based mainly on yield components, (ii) The screening of cultivars-parents and the choice of crossing according to their genotypic profile, proved through a series of criteria and (iii) The selection pattern applied in segregating generations, based on an individual plant performance as a unit of selection and evaluation. Comparable evaluation of progenies requires concurrent selection among and within progenies and application of high selection pressures. This selection procedure reduces genotype x environment interaction and increases heritability. A number of experiments as paradigms have been included throughout the text to better understanding. Breeding assumptions such as decentralized selection and participatory plant breeding have been accepted and incorporated in developing selected cultivars of low-input demands. The text as a whole follows a logical course, from source material, through breeding techniques and selection patterns to maximize heritability and efficiency offering in this way a sound solution in sustainable agricultural problems.

Key words: Analytical breeding, heritability, isolation environment, landraces, selection criteria of F1 and F2

INTRODUCTION

Agriculture is a system of human manipulation of ecosystems to enhance the production of food and fiber in response to social and economic demands and constrains. Sustainability is therefore, the ability of an agroecosystem to maintain its production and functionality through time, in the face of long-term ecological constrains and socioeconomic pressures (Altieri and Merrick, 1987). Humans are an integral part of agroecosystems and a dominant force in determining their current structure. The specific actions by which humans alter these systems are determined by cultural, political, social and economic forces, all of which must be considered as components of agricultural systems. The sustainable agriculture system aims at soil and water conservation, less energy-

intensive modes of production, farm worker safety, protection of prime farm land, preservation of the family farm and enhanced food quality and safety, by managing to penetrate the traditional agricultural policy making establishment (Youngberg and Buttel, 1984). Particular attention is paid to approaches of synthesizing information from different disciplines and applying them to the design of sustainable agriculture systems.

Plant breeding science proceeds by the same mechanisms that have been responsible for the evolution of the wild plant populations and the domestication of cultivated plants since earlier times. The fundamental mechanism is the adaptable change by gene substitution under artificial selection. The objectives and strategies are defined by:

- The agriculture system and the society that is to be served
- The essential genetic features of the crop (Simmonds, 1979)

Therefore, the plant breeder is working towards well defined objectives, economically and biologically reasonable, by developing cultivars that farmers and end users will actually want.

The up to date knowledge in breeding has been progressively leading to a relative uniformity of plant types in comparison with the great overall variation available in the indigenous gene pool (Pecetti and Annicchiarico, 1998). Studies on genetic gain in cereals have shown that, though genetic progress exists at all input levels, genetic gain is lower under low-input levels (Shroyer and Cox, 1993; Ortiz-Monasterio *et al*., 1997; Brancourt-Hulmel *et al*., 2003). This situation may result from breeding conducted either under high- or low-input levels including N fertilizer and fungicides (Brancourt-Hulmel *et al*., 2005). Therefore, cultivar development may need to be geared to the production of genotypes that exploit inputs most efficiently, rather than of genotypes with superior yield only when expensive inputs are needed (Schmidt, 1984; Fasoula and Tokatlidis, 2012). This defines the answer to the question of optimum vs. maximum yield potential.

The present study aims to present the breeding assumptions that support a sustainable agriculture. For that reason, some breeding aspects are summarized hierarchically in the following phases:

- The utility of landraces and old cultivars in modern agriculture
- The tracking and exploitation of the genetic variability of crosses in developing new varieties
- Plant breeding assumptions adapted in a sustainable agricultural system

There is abundant evidence of breeding paradigms in many crops, based on the application of previously acquired knowledge that could offer benefits or solutions to sustainable-agriculture related problems.

FIRST PHASE: UTILITY OF LOCAL CULTIVARS

Breeding landraces or old cultivars is a strategy to improve yield and yield stability in a less favorable agricultural system with lower input levels (Newton *et al*., 2010). Despite earlier predictions about their imminent disappearance (Zeven, 1998), local cultivars still support farming owing to their stability, valuable genes for resistance to biotic and abiotic stresses, quality of the end product and compensation. The extent of the genetic diversity available within them is useful for releasing highest-yielding lines as pure line cultivars, as parents in a crossing program, as multilines or at least in quantifying the adaptive role of specific morphological traits

(Ceccarelli *et al.*, 1987). Their utilization could be envisaged in association with that of indigenous germplasm, largely diversified and very little exploited for breeding.

Yield- and trait-based selection: Focusing on breeding procedures is the most powerful means of achieving higher and more stable yields when crops are subjected either to environmental variability or to sustainable agriculture practices, including the use of low-potential germplasm, or both. The introduction of adapted germplasm from the centers of diversity of crops could be useful to successfully breed (Pecetti *et al.*, 1994). The reason for the choice of germplasm of low potential is to be found in the traditionally low-input farming system and in the better adaptability of these cultivars to the prevailing growing conditions which ensures a fair stability of production (Pecetti *et al.*, 1994). There are two approaches for increasing yield, one is based on increasing yield potential of broadly adapted cultivars, the second relies on the better exploitation of the adaptive features of genotypes by fitting cultivars to specific target environments (Acevedo and Fereres, 1993; Newton *et al.*, 2010).

Direct selection for grain yield potential has traditionally relied on this trait (Annicchiarico and Pecetti, 1988). Traditional breeding is based on either a bulk or a pedigree method of selection (Ceccarelli and Grando, 1997). This procedure is expensive and time-consuming (Ceccarelli *et al.*, 1991). In grain legumes, the application of the Single Seed Descend (SSD) method proved efficient and cost-saving (Haddad and Muehlbauer, 1981). Besides, the phenotypic recurrent selection method allowed breeders to maximize selection progress.

The opportunity to complement yield-based selection breeding with the analytical breeding based on indexes of indirect selection for yield, including sets of morpho-physiological traits, has been put forward, especially for less favorable regions (Richards, 1982), where yield heritability tends to be relatively low (Brawn and Munday, 1982; Annicchiarico and Pecetti, 1998). Analytical breeding may offer tools as a way to increase efficiency of selection (Richards, 1982; Ceccarelli *et al.*, 1991). However, it does not seem appropriate, under the given conditions, as the variable stress level enhances the importance of specific traits under specific situations (Pecetti *et al.*, 1994). It has been found that high yield under less favorable environments is associated with morphological and physiological characters which are different from those associated with high yield under favorable environments (Ceccarelli, 1989). Only the yield components *per se* are constantly associated to grain yield across different environments (Pecetti *et al.*, 1994). On the same basis, a selection index tool proved efficient when applied to several traits at the same time, i.e., when criteria of selection for grain yield included phenotypic measurements of yield components. So, phenotypic parameters as pods per plant, seeds per pod and weight of 100 seeds in common bean (*Phaseolus vulgaris*) increased, after one cycle of selection, by 20% over the base population (Ranalli *et al.*, 1991; Ranalli, 1996). The divergent method of selection was also suggested for establishing the appropriate combination of traits for a given environment (Acevedo and Fereres, 1993).

The description of agronomically important and useful characteristics is a prerequisite for effective and efficient use of germplasm collections in breeding programs (Duvick, 1984). Multi-character indexes of selection are means of considering trait interaction effects in selection decisions. To be of value in assessing a trait in a population of plants, a screening test must satisfy a number of criteria of selection (Ceccarelli *et al.*, 1991; Austin, 1993; Annicchiarico and Pecetti, 1998).

Analytical breeding was predicted to be either as efficient (Atlin and Frey, 1989), more efficient (Calhoun *et al*., 1994), or less efficient (Ceccarelli *et al*., 1992; Sinebo *et al*., 2002) than traditional breeding in the target environment. Analytical breeding has been unsuccessful in the case of:

- Less favorable environments characterized by low yield due to high variability in frequency, timing, duration and severity of a number of climatic variables
- Breeding programs where the major objective is greater yield stability defined as a reduction in the frequency of crop failures (Ceccarelli *et al*., 1991)

Analytical breeding has to consider individual traits as interactive parts of a complicated process rather than in isolation, in order to enhance yield stability in less favorable environments.

One should mention that the factors that interact to influence yielding ability of major field crops are doing so by improving tolerance to higher plant densities in combination with improvements in tolerance to yield potential per plant under low-stress environments (Duvick, 1977). It is therefore, unjustified to breed for genotypes with high yield potential in less favorable environments because most of the time the yield potential cannot be expressed and a much higher priority should be given into improving yield stability. However, in the isolation environment (Fasoulas, 1988; Fasoula and Fasoula, 2000) yield improvement can be quantified independently of the environment by the decrease in the coefficient of variation (CV value), an indication of efficient selection owing to the removal of deleterious alleles. The CV value is the most widely used parameter to quantify variability among individual plants of a crop stand (Edmeades and Daynard, 1979) and a way of estimating yield improvement (Tollenaar and Wu, 1999). In conclusion, this isolation environment suggests that analytical approaches for increasing yield and stability by employing additional yield components (a constitutive character (Ceccarelli, 1989)) may be proved more effective in a selection program.

Adaptability: Studies on the relationships between plant attributes and environmental variables at the collecting sites have been recommended for the evaluation of crop genetic resources (Frankel, 1989). The adaptation of old cultivars to an unpredictable climate is achieved through a highly polymorphic plant community where the maximum fitness at population level is due to different members of the community. The presence within these populations of different types of stress, with their different timing and severity, might explain their yield stability (Ceccarelli *et al*., 1987). Information on the relationships between climatic features at collecting sites and morpho-physiological variation of genetic resources could facilitate understanding of an evaluative adaptation pattern and assist germplasm collectors and users (Annicchiarico *et al*., 1995).

The basic philosophy of those programs is to generate and distribute widely adapted germplasm and to add traits that confer specific adaptation at a later stage (Ceccarelli and Grando, 2002). It was shown for durum wheat (Pecetti *et al*., 1992) and barley (Ceccarelli *et al*., 1991) that some of the material selected under less favorable environments is able to retain its superiority when transferred to a more favorable environment. This superior material constituted about 20 and 30% of the selected genotypes for barley and durum wheat, respectively. A similar proportion (20%) has been observed in durum wheat landraces (Pecetti *et al*., 1994). However, the seed systems associated with commercial agriculture favor a small number of geographically broadly adapted cultivars, as they do not tolerate intracultivar diversity (Berg, 1996).

In local cultivars an understanding of the relationship between the amount of genetic diversity in expressing morphological and agronomic characters and the adaptation to stress environments may elucidate whether the success of landraces to less favorable environments is due to a population buffering mechanism or to a particular architecture of morpho-physiological traits, or both. This may in turn clarify whether "pure line breeding" is the correct approach to breeding for less favorable areas (Ceccarelli *et al*., 1987). Pure line breeding can be successful only if genotypes with a very high degree of phenotypic plasticity are identified.

Direct selection in the target environment is the most efficient strategy (Ceccarelli, 1989). Breeding for specific adaptation is particularly important in the case of crops predominantly grown in less favorable environments, as these tend to be more different from each other than favorable ones (Ceccarelli and Grando, 1997). The relative magnitude of heritability in less favorable and favorable environments is not sufficient to allow a choice of the optimum environment for selection, because phenotypic differences can be of opposite sign in different environments (Ceccarelli, 1989). High yield in very divergent environments appears to be controlled by different sets of alleles across many loci (Cecarrelli, 1994).

Data on the utility of using both an *ex situ* (indirect) and *in situ* (direct) long-term (2004-2008) intrapopulation selection program in the 'Santorini small-sized tomato' landrace (*Solanum lycopersicum*) revealed two homogenous lines, G-line (Fig. 1a) and T-line (Fig. 1b), to co-

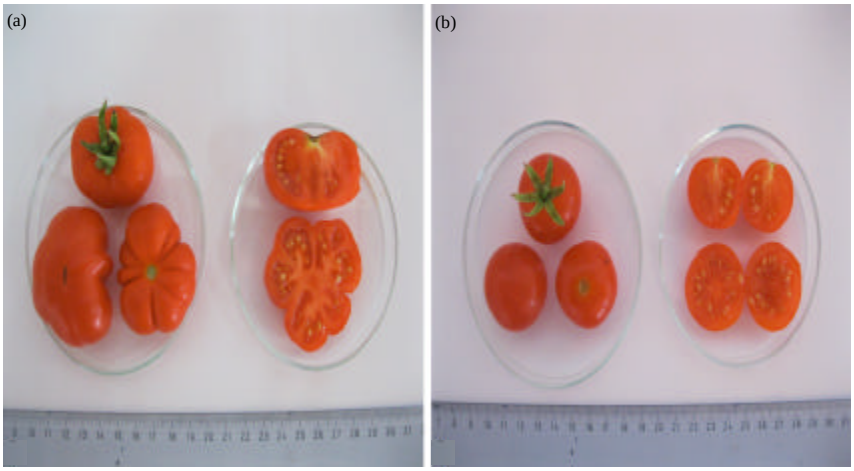

Fig. 1(a-b): (a) Typical fruits of G-line and (b) T-line that co-exist in the landrace 'Santorini small-sized tomato'

Table 1: Spearman coefficient between the fruit yield/plant and the yield components for the 'G' and 'T' lines of 'Santorini small-sized tomato' in the *in situ* and *ex situ* environment

	G-line				T-line			
	Fruit No./plant		Fruit weight/plant		Fruit No./plant		Fruit weight/plant	
Yield components	*in situ*	*ex situ*	*in situ*	*ex situ*	*in situ*	*ex situ*	*in situ*	*ex situ*
Mean early yield	0.77**	ns	0.98**	ns	0.83**	0.81**	0.95**	0.92**
Mean total yield	0.83**	ns	0.94**	ns	0.89**	0.87**	0.96**	0.74**

ns: Non significant values, *Significant correlation at p = 0.05, **Significant correlation at p = 0.01, Dimitriadou *et al*. (2009)

exist. A research was conducted in 2008 involving nine selected families of each line (4th generation). Two experiments (with 420 G-line and T-line plants, respectively) were established *in situ* at Santorini island, under traditional agronomic management, i.e., limited irrigation and seed sowing at hills and two *ex situ* with 312 and 300 plants of each line, respectively, by applying the usual "farmer's technique". A highly significant correlation was shown between yield components per plant and early or total yield in both environments for T-line but only in the *in situ* environment for G-line (Table 1). These data may suggest that:

- The *in situ* evaluation was more reliable
- T-line performed as a broadly adapted line whereas G-line showed specific adaption ability

Some indicative cases of local cultivars/landraces improvement: The value of local cultivars/landraces is mainly attributed to the wide range of genetic resistance against biotic and abiotic stresses, rendering them desirable for adoption in a sustainable agricultural system. In addition, they are characterized by both a broad range of genetic variability, contributing to biodiversity protection and a wide phenotypic variability of useful traits for exploitation in breeding. Furthermore, some of them are in many cases strongly related to the tradition and specific needs of the local people. Usually, local cultivars are cultivated by old farmers in marginal regions and thus being at risk of extinction (Tsivelikas and Koutsika-Sotiriou, 2010). The following cases show how genetic improvement of local cultivars may contribute to sustainability by:

- Pre-breeding techniques for the genotyping of the source material
- Their cultivation revival
- Divergent selection techniques

First case: Pre-breeding techniques genotyping the source material. In the case of *Cucurbita* the use of squash and pumpkin landraces in crop improvement, followed by adaptation, cultivation and marketing of the improved cultivars, has been one of the most sustainable methods to conserve valuable genetic resources and simultaneously to increase agricultural production and food security:

- A pre-breeding research was conducted to study genetic heterogeneity within a *C. moschata* landrace due to the gradual increase of homozygosity and the subsequent detection of inbreeding-tolerant progenies (families) for hybrid breeding (Tsivelikas and Koutsika-Sotiriou, 2010). A different response pattern for each family was revealed for yield and vegetative growth traits due to the increase of homozygosity (Table 2)
- A second pre-breeding research concerned a crossing scheme in two winter squash landraces (*C. maxima* and *C. moschata*) for estimating genetic variance components for yield ability (Tsivelikas, 2010). Generation means analysis was used (Mather and Jinks, 1982). Additive variance was the largest component in *C. moschata* landrace and a three-parameter additive-dominance model accounted for most of the variation among generations of the two landraces (Table 3). However, in some cases, the expansion of the model was necessary, in order to include epistatic effects (Table 3). Heritability estimates indicated low or medium values for

Table 2: Actual model coefficients (b) for traits associated with yield and vegetative growth of a *Cucurbita moschata* landrace and the significance of components of the response curves of each family to the ascending levels of inbreeding

		Yield		Fruit No.		Fruit mean weight		Time to flowering		Seed weight		Pollen grain area	
Family	Response curve	b	t-value[1]	b	t-value	b	t-value	b	t-value	b	t-value	b	t-value
I	Linear	24.6	2.79	ns		ns		0.15	2.62	3.78	2.30	7.90	6.58
	Quadratic	-27.9	-2.48	ns		ns					-11.30	-7.37	
	Cubic			ns		ns							
	Constant[2]	11.3		2.3		3.7		8.57		16.33		12.62	
II	Linear	ns		-4.6	-3.17	ns		0.11	2.52	1.66	2.08	4.89	3.69
	Quadratic	ns		17.1	3.35	ns						-5.58	-3.29
	Cubic	ns		-15.0	-3.34	ns							
	Constant	17.4		2.2		4.9		8.57		12.77		11.48	
III	Linear	-6.9	-2.62	ns		ns		0.14	3.04	1.70	2.61	2.55	6.31
	Quadratic			ns		ns							
	Cubic			ns		ns							
	Constant	14.6		2.1		3.5		8.61		18.94		11.74	

[1]|t|-values 2.145 are significant at p<0.05 and those above 2.977 are significant at p = 0.01, [2]Constant terms for the cases of non significant (ns) responses represent the means of the, respective family (Tsivelikas and Koutsika-Sotiriou, 2010)

Table 3: Genetic effect estimates for traits associated to yield potential for *C. maxima* and *C. moschata* landrace (adapted from Tsivelikas, 2010)

	Genetic effects[1]							
Characteristic	m	(d)	(h)	(i)	(j)	(l)	χ^2	df
C. maxima								
Fruit No.	5.25±0.76	0.85±0.12	-5.30±1.78	-2.00±0.75	-	3.20±1.07		
Total yield	30035±2172	1509,9±356	-15826±5172	-6518±2143	-59.7±1382	10303±3210		
Fruit mean weight	8550± 370.5	-2183±372.6	-411±485.1	-	-	-	4.1	3
C. moschata								
Fruit No.	3.954±0.07	0.679±0.07	-0.75±0.13	-	-	-	3.3	3
Total yield	15938±210.7	2359±209.9	-2220±356.6	-	-	-	2.8	3
Fruit mean weight	3980±262.5	-183±81.2	468±315.8	177±282.4	1167±393.8	-	1.3	1

[1]Symbols for genetic effects are as follow, m: Mean of the parental homozygotes, (d): Additive effects, (h): Dominant effects, (i): Additive x additive epistatic effects, (j): Additive x dominant epistatic effects, (l): Dominant x dominant epistatic effects

C. maxima landrace (H^2 = 0.37-0.54 and h^2 = 0.05-0.37) and medium to high values for *C. moschata* landrace (H^2 = 0.65 – 0.82 και h^2 = 0.39-0.72). As a result, after two cycles of selection, the annual genetic gain for *C. maxima* was 0.86 t h year^{-1} and for *C. moschata* 3.64 t h year^{-1}. Nevertheless, predicted genetic gain for the full-sib families of *C. maxima* was notably lower than that predicted for the respective families of *C. moschata*

Second case: Yield-based selection and analytical selection for cultivation revival of local cultivars/landraces:

- The cultivation revival of a legume landrace (Koutsika-Sotiriou *et al*., 2010). 'Fava Santorinis' (*Lathyrus clymenum*) is a landrace with historical origin, distinct identity, specific adaptability and closely connected with the traditional cultivation system on Santorini island (Hellenic island in the Aegean Sea) (Fig. 2). The breeding procedure lasted five growing seasons (2005-

Fig. 2: Plant of 'Fava Santorinis' (*Lathyrus clymenum*) a landrace with historical origin cultivated under traditional farming system in Santorini island

2009), with the selection based on single plant performance. All experiments were subjected to the sustainable system of cultivation (field rotation, hand weeding, no irrigation and without application of fertilizers and agrochemicals). Improvement of grain yield relied on direct pedigree selection, accomplished by analytical selection for the number of pods per plant, plant leaf color and plant vigor
The breeding procedure succeeded in:

- Increasing the stability of performance of derived populations
- Excluding non productive plants of the original landrace
- Maintaining the mean and variability for pod traits as in the original landrace
- Increasing the grain yield by 42.5-107.9% compared to the original landrace

The heritability of grain yield remained positive throughout the breeding program:

- The cultivation revival of 'Zargana Kavalas', an old snap bean cultivar. This cultivar, registered in the Greek National Catalogues since 1985, is very popular among farmers due to its early maturity and its fine and attractive pod. In the late '90s the cultivar showed a diversion from its original type, related to late maturity and certain pod and seed abnormalities. In order to restore or even improve the original type an intraselection breeding program was applied. The program started with assessing of the existing genetic variability of source material for earliness and pod yield potential. Single-plant frequency distributions for earliness showed that the frequency of unfavorable alleles was high. Thus, the end-target of the breeding program was selection for early maturity retaining and stabilizing high yield. In addition, the seed shape

uniformity was added as a third target. A combined pedigree intraselection program, based on single-plant performance for the aforementioned traits was applied for three successive generations. The evaluation of improved selections and the source material, at dense stand, showed that all selections were producing high and stable early fresh pod harvest, even 53 days after planting (53.25-80.00 g plant^{-1}, compared with 0 g plant^{-1} of the control). Furthermore, the total pod yield of the selections was 219-276% higher than that of source material (Traka-Mavrona *et al*., 2000, 2001, 2002)

Third case: Divergent selection supports and promotes sustainable techniques:

- A dual approach to grafting of melons on *Cucurbita* rootstocks that incorporated breeding and selection for both rootstock and scion, was based on Hellenic landraces, as these were expected to offer adaption to the local specific environmental conditions. Breeding manipulations of the rootstock resulted in improved scion/rootstock combinations with a survival ratio for young grafted seedlings up to 79%, thus upgrading commercially some of the tested *Cucurbita* landraces with limited market value. In addition, the improved scion melon landraces were registered in the European List of Vegetable Cultivars as 'Thrakiotiko', a casaba type and 'Lefko Amynteou', a honeydew type of winter melons (Koutsika-Sotiriou *et al*., 2004)
- Further on, the aforementioned case of divergent selection, applied in melon landrace 'Thrakiotiko' against the soil-borne fungus *Fusarium oxysporum* (f. sp. *melonis* and f. sp. *radicis-cucumerinum*), resulted in the isolation of resistant plants and subsequently in a resistant population derived from them (Giakalis *et al*., 2004). This population was tested as a rootstock in melon grafting along with commercial rootstocks 'Manta' (*C. melo*) and 'TZ-148' (*C. moschata* ×*C. maxima*). 'Thrakiotiko' and the commercial hybrid 'Masada' were used as scions. The results showed that although grafting did not increase significantly early and total fruit yield in treatments with 'Thrakiotiko' and 'Masada' scions, the resistant population of 'Thrakiotiko' as rootstock contributed significantly to the improvement of fruit quality characteristics (°Brix, percentage of dry matter, etc.) in both combinations (Anthimidou *et al*., 2011)

SECOND PHASE: TRACKING AND UTILITY OF GENETIC VARIABILITY OF CROSSES

In general, the breeder sets up crosses in order to generate recombinants, applying criteria of choice to F_1 and F_2 and trying to maximize selection efficiency. Highly heritable characters are affected little by environment and are easily and efficiently selected; characters of low heritability are much more difficult to select. The genetic variability available in a crop always greatly exceeds what a breeder can effectively handle (Simmonds, 1979). Generally, selection in the early generations of a cross, when numbers are large, must be based on efficient criteria, especially for quantitatively inherited characters (Gogas and Koutsika-Sotiriou, 2012). Most of the traits of economic importance are under quantitative genetic control. Subsequently, when numbers have been reduced, actual assessments of yield, yield components, specific disease resistance or quality can be made. Concerns in the second phase are essentially related to the choice of parents, of crossing and of selection patterns of early generations.

Creation of genetic variability: Major breeding concerns are about prediction of potential crosses among parents and of elite crosses, for exploitation. Criteria such as yield performance *per se*, genetic relationship matrix, heterotic relationships, usefulness of derived population

(Lamkey *et al.*, 1995) etc. between any two potential parents are intimately involved and would aid the breeder in identifying from among a large number of crosses those most likely to yield the most productive lines, thus saving time and effort.

A number of studies (Atkins and Murphy, 1949; Fowler and Heyne, 1955; Lupton and Whitehouse, 1955) have shown that the poorer (rejected) crosses sometimes produce superior lines. On the other hand, lines of crosses identified as high yielding in F_1 had significantly greater mean yield in F_4 than lines of crosses that were low yielding in F_1 (Nass, 1979). Many breeders (Bhatt, 1973; Jensen, 1988; Lupton, 1961) used a diallel cross technique to assess the usefulness of parents and early-generation progeny performance for identifying the most promising crosses. A common procedure, heterosis over mid-parent value and heterosis over best-parent value as a criterion for identifying parental combinations capable of producing the highest level of transgressive segregants has also been applied (Roupakias *et al.*, 1997; Gouli-Vavdinoudi and Koutsika-Sotiriou, 1999; Singh *et al.*, 2004; Kotzamanidis *et al.*, 2008). Panter and Allen (1995) suggested using best linear unbiased prediction method to identify pairs of lines with high mid-parent values and to select among such pairs those that were the most genetically diverse based on the relationship matrix of traits or genetic markers.

It has been pointed out (Hinson and Hanson, 1962; Khalifa and Qualset, 1974; Shebeski and Evans, 1973) that the adverse effects of competition on testing early generations obscured the evaluation data. Fasoulas (1988) proposed and others, Fasoulas (1977) Fasoula and Fasoula (2000) Kotzamanidis *et al.* (2008) and Gogas and Koutsika-Sotiriou (2012) applied the evaluation of widely-spaced plant performance of F_1 and F_2 in honeycomb designs. Following this testing procedure in wheat the F_5 lines, derived from high-yielding crosses, significantly exceeded the highest yielding line of the lowest-yielding cross by 25-31% while in a solid stand, this percentage ranged from 25-40% (Gouli-Vavdinoudi and Koutsika-Sotiriou, 1999).

In some crops such as maize, tomato, etc. the single cross hybrids (F_1) constitute the commercial cultivars. In this case a pattern of crosses of source material supports breeder's evaluation. So, source germplasm used by maize breeders for inbred lines development, included primarily F_2 (elite x elite inbred line crosses), backcross and synthetic populations (Bauman, 1981).

Publicly and privately funded breeding programs have contributed to genetic improvement of maize hybrids in the United States. Evidence from the Fountain and Hallauer (1996) study suggests that average genetic variability within F_2 populations exceeds that within narrow-base synthetic populations. However, F_2 is the most commonly used parental germplasm (Bernardo, 1996). Thus, breeder's interests have been focused on relatively short-term projects for the improvement of narrow-base populations composed of elite germplasm. An approach for the evaluation of different germplasm based on a pattern of crosses includes:

- Selfing of F_1 and F_2 and subsequently estimating the F_2 tolerance to inbreeding depression
- Diallel crosses between hybrids, estimating the general combining ability
- Test crosses between hybrids with an elite inbred line, estimating the specific combining ability Thus, the single cross hybrid that is possible to develop elite inbred lines quicker than others is defined (Koutsika-Sotiriou, 1999; Koutsika-Sotiriou and Karagounis, 2005)

These studies took into account:

- The various types of gene actions indicating that complete dominance is much more important than overdominance in the improvement of maize hybrids (Sprague and Eberhart, 1977; Jenkins, 1978)

- The fact that inbreeding depression is mainly caused by the fixation of undesirable mutant genes preserved in hybrids by heterozygosity (Fasoulas, 1988)
- That the components of variance for maize lines with general combining ability are mainly additive, whereas the components of variance for lines with specific combining ability are dominant (Sprague and Eberhart, 1977)

Hence, combining ability is highly related to heterosis connected to the presence of deleterious genes (Fasoulas, 1988) and thus a hybrid with low inbreeding depression, positive general combining ability and negative specific combining ability deserves the preference of breeders.

In a horticultural crop where the use of F_1 tomato hybrid cultivars has increased, the assessment of source material followed a procedure similar to the one used for the assessment of maize source material i.e., (i) Selfing of F_1 (ii) Diallel crossing between hybrids or cultivars, an indication of General Combining Ability (GCA) (iii) Testcrosses of hybrids with testers, an indication of Specific Combining Ability (SCA) and additionally (iv) removal of undesirable traits (Koutsika-Sotiriou and Traka-Mavrona, 2008; Koutsika-Sotiriou *et al*., 2008).

The flux of parental material in any breeding program, private or public, is based on a working strategy, known as "the assessment of the continual turnover of the cultivars". As older parents retreat, new ones enter from adapted cultivars and recombinant lines resulting from F_2 of elite hybrids. The suggested pattern of crosses takes into account the heritability of "general worth" and thus provides cultivars with lower demands that may be able to confront less favorable environments (Simmonds, 1979).

Genotyping and phenotyping: To utilize the potential of each genotype the breeder needs the appropriate data of each phenotype which can be easily interpreted, in order to transform it in a powerful tool to improve yields in less favorable environments (Ceccarelli, 1996). The main contribution of breeding to sustainability is to: decrease genotype x environment interactions, increase heritability through selection and repeatability through cultivar evaluation. An approach towards genotype x environment interactions is to provide methods for separating genetic effects from environmental effects, i.e., to breed for maximum yield and stability in macroenvironments (Ceccarelli, 1989). Therefore, it is not the relative magnitude of heritability in a favorable or less favorable environment but the extent to which the differences among genotypes, observed under a given environment, are maintained when the same genotypes are compared in a different environment (Ceccarelli, 1989). Breeding methodologies need to address the potential for large genotype x management system interactions, sometimes caused by altered agronomic practices (Rosielle and Hamblin, 1981). If this is the case, then: (i) breeding methodology developed for a given crop may not be applied unreservedly to different crops, i.e., the genotypic variance in wheat under the favorable environment was several times greater than in a less favorable one while in barley differences were much smaller and (ii) breeding methodologies develop more robust, broadly-adapted cultivars that may need to be selected in order to perform well across different management systems. Using wheat cultivars it was pointed out (Trethowan *et al*., 2005) that studies comparing grain yield under various tillage systems are scarce and that the cultivars tested were developed using conventional tillage practices, i.e., there is a need for further work on genetic gain in wheat breeding programs (Richards *et al*., 2006). Focusing on Ceccarelli's statement (Ceccarelli, 1989), to succeed genotyping through phenotyping, the key is the difference among genotypes to be maintained under different environments.

THIRD PHASE: SELECTION PATTERNS AND BREEDING ASSUMPTIONS ADAPTED FOR A SUSTAINABLE AGRICULTURAL SYSTEM

Conventional breeding, with the inbred-hybrid cultivar system is still remaining the most important breeding scheme for the development of commercial cultivars (Miranda Filho, 1999). This breeding scheme within modern agriculture has resulted in the narrowing of the genetic base, in vulnerability to biotic and abiotic stresses and in limiting future gains from selection (Tailer and Bernardo, 2004). In other words, the liberal use of high inputs, the loss of genetic diversity and the stagnation of yields in less favorable areas are the negative consequences of modern agriculture and conventional breeding (Annicchiarico and Pecetti, 1988; Tilman, 1996). Today, the pressure to move towards a sustainable agriculture creates the necessity to:

- Have access to, exploit and incorporate a broader germplasm (topics that are discussed in the First and the Second Phase in this manuscript)
- Develop cultivars specifically adapted to changing farming systems, focusing on heritability of general worth during selection procedure

Genotyping and phenotyping through selection: Plant breeding is not a discipline immediately connected with agricultural sustainability, as sustainability is associated with farming practices (Richards *et al*., 2006). Practices as crop rotation, reduction of inputs, particularly nitrogen fertilizers, etc., decrease the genetic variance and thus the efficiency of selection. Techniques to maximize genetic gain in crop yield include selection for both yield performance and stability. These screening techniques which are field-oriented are usable and preferable. Heritability estimates usually have high standard errors unless based on very large samples (Falconer, 1981) and heritability is higher in favorable than in less favorable environments (Blum, 1988). It was shown Rosielle and Hamblin (1981) that each one of several loci, indirectly controlling the expression for yield under stress, is different from those controlling the expression of yield under non-stress environments. Recently, the concept of the whole-genome phenotypic evaluation recognizes that genes controlling crop yield concern the genome as a whole and belong to three categories (Fasoula and Fasoula, 2002, 2003): (i) genes that control yield potential per plant, estimated by the progeny mean ($\overline{X}$) (ii) genes that confer tolerance to abiotic and biotic stress, estimated by the progeny standardized mean ($\overline{X}$/s) and (iii) genes that control responsiveness to inputs, estimated by the progeny standardized selection differential ($\overline{X}$ sel-$\overline{X}$)/s). Effective selection for the genome as a whole leads to the development of cultivars combining high and stable productivity (Fasoula and Fasoula, 2002). The use of the three components of crop yield potential, regulated by the three gene categories, leads to the development of density-independent cultivars (Fasoula and Fasoula, 2000; Tokatlidis *et al*., 2001). Therefore, cultivars selected for the three components of crop yield will perform optimally under a wide range of plant densities. In addition, evaluation of plants in the absence of competition promotes rapid early growth that facilitates suppression of weeds. Honeycomb designs maximize efficiency in selection by: (i) preventing the masking effects of competition thus identifying heritable superiority and (ii) maximizing phenotypic expression and differentiation (Fasoulas, 1973, 1988, 1993). The prospects of the honeycomb methodology are to support the sustainable agriculture because the approach and the evaluation of genotypes are based on eco-breeding principles (Van Bueren *et al*., 2003).

Some indicative cases of applied heritability of general worth in segregating generations: Currently, the need for increased sustainability of performance in cultivars is a socio-political demand. Efforts to compare the outcome of breeding programs under favorable and less

favorable environments are not only a challenge but a necessity. The next two cases concern the evaluation of the outcome of breeding programs conducted under such conditions.

The first case refers to a breeding program of the landrace 'Fava Santorinis' (*Lathyrus clymenum*). It was a long-term (2005-2009) program with indirect intra-landrace selection. Fourth-generation progenies compared for grain yield *in situ* and *ex situ* with the source landrace was shown to be superior by 42.48-107.89% (Koutsika-Sotiriou *et al*., 2010). Moreover, the distance (Ouyang *et al*., 1995), between the two environments was found low (equal to D = 0.02), indicating that the *in situ* and the *ex situ* environments were close enough to interact with the genotypes. This is an indication that the performance of the selected populations in the *ex situ* environment was equal to that in the *in situ* one. In this sense the indirect selection "succeeded" in carrying the genetic gain over to the *in situ* environment (Dawson *et al*., 2008). This was feasible because the high heritability of traits in this case combined with the low genetic correlation coefficient between the two environments.

The second case refers to a breeding program applied to tomato cultivars for fresh consumption. Two different gene pool sources were used in an experiment, i.e., local cultivars (seven open-pollinated landraces, belonging to the Hellenic Gene Bank, contact: kgeggb@otenet.gr) and five recombinant lines from the commercial single-cross hybrids 'Iron' and 'Sahara'. These two cultivar sources were evaluated under conventional and organic farming system. The typical cultivation practices were followed, i.e. crop rotation with legumes, manure, soil mulching by biodegradable film and no fertilizer and agrochemical applications. Plant performance showed that under the conventional system fruit yield was increased while under the organic system fruit yield was decreased while the discrimination among entries was increased. Therefore, the cultivars with high productivity and stability of performance were prominent. The recombinant lines, although developed under a conventional system, showed a well-adapted performance both under conventional (Table 4) and under sustainable (Table 5) farming system, proving that the applied

Table 4: Early and total fruit yield (fruits/plant and g/plant) of tomato landraces and recombinant lines (Ir- and Sah- codes) in the conventional system of cultivation

	Earliness		Total yield	
Entry	Fruit No./plant	Fruit weight/plant (g)	Fruit No./plant	Fruit weight/plant (g)
Makedonia	8.94^{abc}	1453^{a}	16.78^{bcd}	2702^{a}
Milo Chalkidikis	6.75^{bcd}	1125^{abc}	16.31^{bcd}	2659^{a}
Milati Kefalonias	11.22^{a}	1438^{a}	23.78^{a}	3197^{a}
Pantaroza	11.58^{a}	1389^{ab}	26.06^{a}	2983^{a}
Souvritiki Evrou	5.75^{bcd}	822^{abcd}	12.58^{de}	1802^{a}
Milo Serron	8.42^{abc}	1410^{ab}	14.94^{cd}	2554^{a}
Bull Heart	1.53^{e}	269^{d}	13.28^{d}	2638^{a}
Milo Corfu	2.75^{de}	846^{abcd}	6.83^{e}	1986^{a}
Ir-M-F_8	4.97^{cde}	693^{cd}	15.06^{cd}	2156^{a}
Ir-HS-14	4.92^{cde}	738^{abc}	14.28^{cd}	2137^{a}
Ir-HS-17	9.17^{ab}	1268^{abc}	14.94^{cd}	2101^{a}
Sah-HS-17	8.47^{abc}	859^{abcd}	20.86^{abc}	2354^{a}
Sah-HS-20	11.36^{a}	1372^{abc}	22.03^{ab}	2566^{a}
CV (%)	29.71	33.74	21.74	24.16

Values with the same letter within a column are not significant differences according to Duncan (p = 0.05) (adapted from Avdikos *et al*., 2011)

Table 5: Early and total fruit yield (fruits/plant and g/plant) of tomato landraces and recombinant lines (Ir- and Sah- codes) in the organic system of cultivation

	Earliness		Total yield	
Entry	Fruit No./plant	Fruit weight/plant (g)	Fruit No./plant	Fruit weight/plant (g)
Makedonia	5.93^{de}	770^{bc}	8.80^{cd}	1084^{bcd}
Milo Chalkidikis	6.87^{bcd}	873^{abc}	13.17^{b}	1573^{ab}
Milati Kefalonias	6.53^{cd}	636^{bcd}	11.43^{bc}	1101^{bcd}
Pantaroza	8.70^{abc}	760^{bc}	12.83^{b}	1230^{bcd}
Souvritiki Evrou	2.57^{f}	330de	4.07^{ef}	529^{ef}
Milo Serron	5.40^{de}	852^{abc}	8.00^{cd}	1181^{bcd}
Bull Heart	3.57^{ef}	565^{cd}	5.47^{de}	813de
Milo Corfu	0.27^{g}	59^{e}	0.97^{f}	279^{f}
Ir-M-F_8	5.03^{de}	605^{bcd}	8.87^{cd}	1010^{cd}
Ir-HS-14	7.13^{bcd}	913^{ab}	15.13^{ab}	1838^{a}
Ir-HS-17	9.10^{ab}	1104^{a}	14.27^{b}	1722^{a}
Sah-HS-17	6.83^{bcd}	671^{bc}	13.93^{b}	1404^{abc}
Sah-HS-20	10.97^{a}	1156^{a}	18.33^{a}	1885^{a}
CV (%)	22.23	23.45	20.95	22.08

Values with the same letter within a column are not significant differences according to Duncan (p = 0.05) (adapted from Avdikos *et al*., 2011)

cultivation system (conventional or not) and the adaptability of lines/landraces (adapted or not) had inferior significance to a breeding procedure where selection of "general worth" was applied. Conclusively, a sustainable breeding scheme for a conventionally cultivated crop as the tomato seems to facilitate selection of superior genotypes, as the expression of favorable traits is higher and fixation of additive genes can be accomplished successfully. The results also showed that stable hybrids incorporate individual buffering ability into inbred line cultivars (Avdikos *et al*., 2011).

ADAPTED BREEDING METHODOLOGY AND CONSEQUENCES TO SUSTAINABILITY

Taking into consideration the previous cases, some additional assumptions ought to be made. Breeding for sustainability moves the centralized breeding programs to largely decentralized ones. The term "decentralized selection" (Simmonds, 1984) is defined as selection in the target environment associated with specific adaptation to a less favorable environment. The most serious limitation of decentralized selection in less favorable environments is the large number of target environments which calls for a larger sample of selection environments and farmers' participation. The participation of farmers in a breeding procedure is known as "participatory plant breeding". Decentralization is the major contributor to improved biological results of plant breeding while participation contributes to outcomes, i.e., to cultivars derived through it (Ceccarelli and Grando, 2002).

The participatory plant breeding corrects a common inefficiency in breeding programs because the selection and release of cultivars can meet farmers' requirements and needs. Information about farmers' selection criteria is highly important for participatory plant breeding (Ceccarelli and Grando, 2002). Usually, farmers are interested in a wider range of traits related not only to adaptation to various growing environments but also to marketability. Farmers, although rank yield as their most important selection criterion, they in fact select for several other traits as long as yields are above an acceptable minimum (Ceccarelli and Grando, 2002). Farmers' selection criteria for traits such as taste, color, cooking properties and nutritional value may differ from those

of breeders (Sperling *et al.*, 1993). Participatory plant breeding and cultivar selection have been proven more successful than the approach used in high-input breeding programs for cultivar improvement in stress-prone environments where sustainable approaches rank high (Newton *et al.*, 2010).

CONCLUSION

The contribution of plant breeding in sustainable agriculture will be in providing germplasm for these new practices and devising new methods of selection (Richards *et al.*, 2006). The present review is about more efficient breeding methods that increase crop productivity, stability and responsiveness to water and nutrient use by the selected genotypes, either in a local cultivar or in the segregating offsprings of a cross. Genotyping and phenotyping accuracy is succeeded by continuous exploitation of adaptive or newly created variation under an isolation environment (Fasoulas, 1988; Fasoula and Fasoula, 2000, 2002, 2003). Tracing adaptive variations which stem from the interaction between genotype and environment (Goldberg *et al.*, 2007) allows profitable exploitation of resources and continual incorporation of gene variants for upgrading local cultivars. In that way plant breeding succeeds in increasing heritability of "general worth" by improving the precision with which genotypes are selected and contribute to improvements in sustainable-agriculture farming.

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