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# Development of crop cultivars by honeycomb breeding

Vasilisa A. Fasoula · Ioannis S. Tokatlidis

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**Abstract** The ability of agriculture to adapt to environmental changes and to address main issues of food quality and environment protection is a fundamental factor in achieving sustainability. Low yield capacity of contemporary sustainable farming systems, however, is a major obstacle to future growth of sustainable agriculture. In addition, increasing pressure is placed for higher food supply due to the projected population increase. To overcome these barriers and stimulate the wide adoption of sustainable agriculture, ample supply of cultivars that satisfy the requirements for sustainability without compromising productivity is essential. Otherwise, the viability of sustainable agriculture is unsound. Moreover, plant breeding has to be a non-stop process supporting agriculture because of the ongoing climate changes. The studies of the effects of competition on crop yield and selection efficiency unravelled important findings for plant breeders. Firstly, the uppermost cultivar type is the mono-genotypic and particularly the highest evolutionary grade of ‘pure line’. Secondly, single plant selection is effective only when it is realized in the absence of competition for growth resources. Honeycomb methodology, by considering as a major principle the application of selection in the absence of competition, counteracts the disturbing effects of competition on selection effectiveness. Furthermore, the

honeycomb experimental designs cope with the confounding implications of soil heterogeneity. These two findings help breeders to consider the individual plant as an evaluating and selection unit. As a consequence, the development of pure line cultivars that fully meet the needs of a sustainable agriculture is possible. Most importantly, honeycomb breeding exploits effectively not only favourable but marginal environments as well through the development of density-neutral cultivars. Marginal environments are exploited optimally when lower plant populations are used. It is of essence to realize that without the ability of exploiting successfully marginal environments which represent the majority of the production environments globally, sustainability in agriculture becomes problematic.

**Keywords** Buffering · Competition · Homeostasis · Honeycomb breeding · Monoculture · Resource use efficiency · Response to selection

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## 1 Introduction

Conventional agriculture systems of production have been criticised for large amounts of external inputs, soil erosion and degradation; chemical contamination using genetically homogeneous cultivars; and loss of biodiversity (Malézieux et al. 2009; Rodriguez et al. 2009; Zhang et al. 2009). In turn, there is now more scepticism and concern that we should be more oriented towards a precautionary approach of agricultural practices. A widespread consensus exists about the importance of sustainable agriculture in economic planning and human development (Xu et al. 2006; Gafsi et al. 2006; Lichtfouse et al. 2009). Despite the lack of absolute consensus on the meaning of sustainable agriculture, there is an overall agreement on its multidimensional characteristic, with environmental, economic and social approaches integrated in the sense of sustainable agriculture (Shaller 1993; Kruseman et al. 1996; Kropff et al. 2001; Gafsi et al. 2006; Lichtfouse et al. 2009).

Even though the efficacy of agriculture systems conducive to economic, environmental and social sustainability of farming operations has been demonstrated, the adoption of sustainable agricultural practices is not widespread (Rodriguez et al. 2009). The concept of sustainability has yet to be made operational in many agricultural situations, but successful conditions requested for the implementation of sustainable agriculture remain a difficult issue (Gafsi et al. 2006). According to Rodriguez et al. (2009), the producers' belief that conventional systems give higher yields was identified as the biggest barrier to adopt sustainable agricultural practices.

A major cause of inability of sustainable agricultural systems to arouse enthusiasm is the lack of appropriate cultivars for the specific requirements of sustainable agriculture. Efforts to diffuse improved cultivars obtained by the first green revolution have had considerable success; however, a new transformation is needed emphasizing environmental-friendly agricultural practices (Blackman 2000). Food demand globally is expected to double or even triple by 2050 (Green et al. 2005; Gepts 2006; Gowing and Palmer 2008; Stuber and Hancock 2008). Hence, implementation of sustainability into farming systems cannot ignore the magnitude of the requested increase in food production. Moreover, agriculture is one of the sectors most vulnerable to the risk and impacts of global climate change (Tingem et al. 2009), so the development of new cultivars adaptable to ongoing environmental changes is an imperious need.

So far, plant breeding has been the most important contributor to phenomenal yield increases and the development of sustainable both biotic and abiotic stress resistance in many crop and horticultural plants (Stuber and Hancock 2008). Plant breeding started as a science soon after the rediscovery of Mendel's laws, has evolved over the years allowing the exploitation of genetic resources more thoroughly, and has remained a vibrant science with continuous success in developing and deploying new cultivars on a worldwide basis (Gepts and Hancock 2006). At present, plant breeding orientated towards the major principles of sustainable agriculture has the potential of overcoming the particular impediment of suitable for sustainable agriculture cultivars and, combined with other appropriate strategies, might be proven a fruitful parameter for the future expansion of sustainable agricultural practices.

Depending on the crop, the cultivated cultivars are either mono-genotypic like pure (inbred) lines, single-cross hybrids and clones or poly-genotypic such as open-pollinated populations, multi-line mixtures and synthetic cultivars. Plant breeding targets towards developing new cultivars characterized by improved agronomic attributes. Agronomically important quantitative traits, i.e. improved yield, stability and quality, are usually the primary breeding goals whether the product harvested is seed, forage, fibre, fruit, tubers, flowers, or other plant parts (Sleper and Poehlman 2006). Breeding effectiveness for these traits depends on the precision with which the genetic potential of individuals or families is assessed. The assessment of the genetic potential for quantitative traits is subject to errors of various causes such as environmental heterogeneity in the selection field and lack of precision in the process of trait measurement (Yano et al. 2002). The genotype-to-phenotype gap certainly still exists, and the challenge is to design experiments that make the best use of the resources needed to bridge it (Wilson et al. 2003).

Resource use efficiency, being of prime importance in terms of sustainability, is heavily reliant on competition among individuals within a plant population. On the other hand, plant breeding has not seriously considered the effect of competition on single-plant yields, although competition exerts confounding effects on the identification and selection of the superior genotypes, contributing thus to the genotype-to-phenotype gap. However, an innovative breeding procedure has been established, namely the honeycomb methodology (Fasoulas 1988, 1993; Fasoula and Fasoula 1997a, b, 2000, 2002), which places particular emphasis on the issue of competition. Accordingly, this chapter focuses on (1) the role of competition on crop resource use efficiency and the reflected type of cultivar, (2) the major breeding principles related to competition that enhance selection efficiency and (3) the honeycomb breeding methodology as a breeding procedure to develop cultivars suitable for the conditions of sustainable agriculture.

## 2 The role of competition in the resource use efficiency

The genetic and environmental factors responsible for the genotype-to-phenotype gap, and accordingly for the gain stagnation in many crops, have been thoroughly explained (Fasoulas 1988, 1993; Fasoula and Fasoula 1997a, b, 2000, 2002). Herein, two major topics related to these factors are discussed, aiming to emphasize on principles underlying the selection efficiency and the development of successful cultivars. The objective was to place particular emphasis on (1) which kind of cultivars, i.e. mono-genotypic or poly-genotypic, the breeding strategy should be orientated towards and (2) the elucidation of the conditions that optimize response to selection. From this viewpoint, there have been crucial insights gained by the definitions firstly of the possible competition conditions at which a given genotype may be evaluated (Fasoula and Fasoula 1997a, 2002) and secondly of the evolutionary course of crop plants under domestication (Fasoulas 1993; Fasoula and Fasoula 1997b, 2002).

### 2.1 The concept of competition and competitive ability

Competition has been defined as the plant-to-plant interference with the equal use of density-limited underground and aboveground growth resources, e.g. water, nutrients and light. Unequal use of resources under competition is due to competitive advantages of some plants over others. Competitive advantages and disadvantages are either genetically or environmentally induced, resulting in increased differences in growth and development of plants within the stand because of uneven growth suppression (Fasoula and Fasoula 1997a, 2002).

Genetically induced advantages and disadvantages are due to differences among plants for ‘genetic competitive ability’ within a genetically heterogeneous population grown under density-limited resources. Strong genetic competitive ability of a plant means that its genotype allows it to get more resources than its share against the less competitive neighbours. Individual plants with such a capacity get progressively competitive advantage over the weak competitors, and thus growing conditions become more and more favourable for the former and more and more adverse for the latter.

Environmentally induced advantages and disadvantages originate from acquired variance within the stand population. Acquired variance is very common even within genetically homogeneous stands due to various causes. A variation in seed emergence comes from differences in sowing depth, seed size and soil texture; failure to access soil water; effects of clods and capping in wet soils; and insects, diseases, birds and rodents (Pommel and Bonhomme 1998). After emergence and during plant growth, plant-to-plant variability is broadened due to age differences, environmental heterogeneity, differential effects of herbivores, parasites or pathogens, and, in most cases, because of interactions among these factors (Pan et al.

2003). In turn, plant-to-plant variation is created, and a number of plants get ‘acquired competitive ability’ and growth advantage over their less vigorous neighbours, getting the ability to obtain more resources than their share and to suppress the growth of smaller individuals.

Competitive ability along with resource limitation determines the competition intenseness within a plant population. Whilst competition originating from the genetic competitive ability is inevitable, the one due to acquired competitive ability can be optimized. Fasoula and Fasoula (1997a) have defined three categories of competition where a given genotype may be evaluated. These categories, illustrated in Fig. 1, are (1) the ‘isolation environment’, (2) the ‘crop environment’ and (3) the ‘competition environment’.

#### 2.1.1 The isolation environment

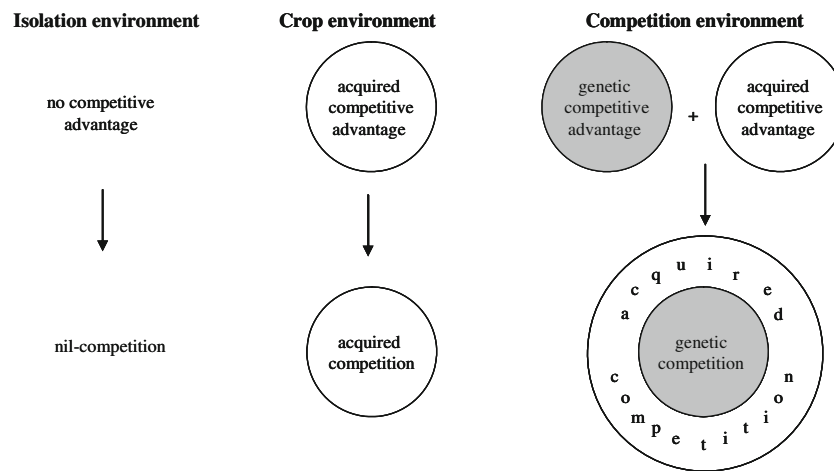
In the isolation environment, individual plants are spaced so widely apart as to eliminate any plant-to-plant interference for the equal use of growth resources (Fasoula and Fasoula 1997a). Because individual plants are not affected by the competitive ability of neighbouring plants, the condition is deemed as ‘nil-competition’. So every plant in the stand is reliant solely on its own genetic potential throughout the whole developmental cycle, from emerge to the reproductive stage. Therefore, the isolation environment assesses accurately the full genetic potential of single plants for all the traits measured (Fasoula and Fasoula 1997a, 2002; Tokatlidis et al. 2010a).

#### 2.1.2 The crop environment

In the crop environment, the genotype is in competition with itself and identical genotypes theoretically share environmental resources equally, so their yield is evenly suppressed (Fasoula and Fasoula 1997a). The crop environment occurs in monoculture systems of the farmer’s field, composed of mono-genotypic cultivars, like pure lines, single-cross hybrids and clones. In the crop environment, a number of plants get acquired competitive ability and growth advantage over their less vigorous neighbours, being able to get more resources than their share and suppress the growth of smaller individuals. Consequently, environmentally induced competition is developed, the scale of which depends on the crop species, the cultivar and the measures taken to reduce it. The crop and cultivar genetic background (buffering) is of prime importance reflecting genotype vulnerability to the exogenous forces responsible for acquired variance.

#### 2.1.3 The competition environment

In the competition environment, the genotype is in competition with genetically different genotypes. Available resources are shared among dissimilar genotypes unequally due to their



**Fig. 1** Causation of competition within a plant population stand (Fasoula and Fasoula 1997a): (1) in the isolation environment, large interplant distance excludes competitive advantages and disadvantages, so competition is zero; (2) in the crop environment, thanks to genetic homogeneity, only environmentally induced variation exists;

thus, acquired advantages and disadvantages end up to acquired competition; and (3) in the competition environment, genetically plus environmentally induced variation exists, so both genetic and acquired advantages and disadvantages end up to heavy competition

different genetic competitive ability, so the yield of individuals is unevenly suppressed (Fasoula and Fasoula 1997a). The competition environment occurs in the farmer's field when polygenotypic cultivars are grown, like populations, cultivar mixtures, etc. More importantly, competition environment occurs within sampled materials in the various generations of selection grown under dense stand conditions. Competition in this case originates from both genetic and acquired differences. Thanks to their genetic competitive ability, some plants have a good chance to obtain acquired competitive ability as well. Hence, the gap of competitive advantage and disadvantage among plants is enhanced, and thus strong interplant interferences with equal sharing of resources prevail. In brief, competition is stronger in the competition than in the crop environment due to its double nature.

Two logical questions arise from the competition specifications mentioned above: (1) Is the crop or competition environment preferable in the farmer's field? (2) Does the competition or the isolation environment approach optimal conditions to establish a breeding process? The answers to these questions have been clarified in detail firstly in the reviews by Fasoula and Fasoula (1997a, b, 2000, 2002) and will be further discussed in the following sections.

## 2.2 Crop versus competition environment

### 2.2.1 The evolution pattern through domestication and crop buffering

Relevant to the first question is the pyramidal evolution pattern where crops are categorized into four main groups (Fasoulas 1993; Fasoula and Fasoula 1997b, 2002, 2005). The higher the position of a crop in the pyramid, the higher its position in the

evolutionary scale, the smaller the load of deleterious genes it carries and, thus, the higher the degree of its genetic buffering. Accordingly, the position of a crop in the pyramid reflects the effort required to improve the crop.

Pure line cultivars in autogamous crops, such as tomato, cotton and soybean, are the one extreme of the mono-genotypic cultivars (Fasoulas 1993; Fasoula and Fasoula 1997b, 2002). They are positioned at the top of the pyramid because they carry the lowest load of deleterious genes. In this group, continuous selfing and natural or artificial selection allowed homozygosity to exploit favourable additive gene action, accompanied by a simultaneous gradual removal of deleterious genes. Predominance of pure lines in this group is attributed to the increased amount of the gene product due to additive homoallelic complementation, leading to the so-called inbred vigour (Fasoula and Fasoula 2002, 2005).

The second group consists of crops whose reproductive system favours cross-fertilization, and the breeding effort led to the predominance of the other extreme of mono-genotypic cultivars, the single-cross hybrids (Fasoulas 1993; Fasoula and Fasoula 1997b, 2002). A good representative is maize, the crop evolution of which throughout the twentieth century deserves particular consideration. Data presented by Troyer (1996) showed that in the USA, until the 1930s, open-pollinated populations were cultivated and the annual gain in grain yield approached only 1 kg/ha. The cultivation of the less heterogeneous double-cross hybrids during the period from 1930s to the 1960s enlarged the annual gain to 65 kg/ha. Since then, the appearance of the mono-genotypic single-cross hybrids blew up the annual gain to 110 kg/ha. The advancement in maize through the route 'population→double-cross hybrid→single-cross hybrid' signifies the progressive elimination of deleterious genes and the parallel

transition from poly-genotypic to mono-genotypic cultivars (Fasoula and Fasoula 2000, 2002, 2005). In maize, a considerable amount of deleterious genes still exists, and the effects of these genes can be masked in single-cross hybrid cultivars through either dominant genes or through trans-complementation in the case of pseudo-overdominant alleles (Fasoulas 1993; Fasoula and Fasoula 1997b, 2002, 2005; Tokatlidis et al. 1999, 2008a).

The third pyramidal level is occupied by crops for which heterogeneous populations are still cultivated, like alfalfa. Finally, at the bottom of the evolutionary scale are asexually reproduced crops with the predominant cultivar type being clones, e.g. potato. These two crop groups carry the heaviest load of deleterious genes, therefore requiring more breeding effort for their improvement (Fasoula and Fasoula 1997b, 2002).

Intensive breeding efforts throughout the twentieth century to improve the yield potential of crops were generally successful. However, all of the successfully improved crops were inbreeders, e.g. wheat, or outbreeders characterized by the predominance of additive genetic variation, e.g. maize (Fasoula and Fasoula 2002). In contrast, in the clonally propagated potato, selection for productivity has failed to establish genetic gain (Douches et al. 1996). The principal cause for the reported yield stasis has to be explored in the high load of deleterious genes that are locked in repulsion phase linkages and are responsible for the high degree of degeneration following gene fixation (Fasoula and Fasoula 2002). Alfalfa is another crop that has failed to establish genetic gain through selection because of the high load of deleterious genes. Indeed, the genetic contribution to yield in alfalfa over the period from 1898 to 1985 was only 3% (Holland and Bingham 1994).

The ultimate breeding goal of pure line cultivars becomes feasible even in non-autogamous crops by the systematic removal of deleterious genes and their replacement by favourable additive alleles (Fasoulas 1993; Fasoula and Fasoula 2000, 2002, 2005). In maize, there is overwhelming evidence on the predominant role of dominance and partial dominance in the control of heterosis (Crow 2000), suggesting a possibility of developing inbred lines able to reach hybrid productivity levels (Fasoula and Fasoula 2002, 2005). Elimination of deleterious genes and accumulation of favourable alleles via inbreeding and selection was found to improve forage yield by 24% even in an alfalfa population (Kimbeng and Bingham 1998).

Obviously, pure line cultivars offer two advantages that are of paramount importance for the development of sustainable agriculture: (1) they breed true to type and have low cost of certified seed production and (2) producers may retain their own seed for the next season, on the presupposition of isolated propagation in the case of cross-pollinators. This is essentially important for poor small-scale farmers in developing countries. Moreover, the homozygotic structure of pure line cultivars enables them (3) to display high and stable yield and (4) to exhibit high

tolerance to biotic and abiotic stresses supposing they carry favourable additive genes.

### 2.2.2 Cultivar buffering

In mono-genotypic cultivars, the phenotypic variance represents absolute environmentally induced variance. Accordingly, when different homogeneous genotypes are tested under comparable conditions in pure stand, differences in phenotypic variation reflect their vulnerability to variance induced by non-genetic factors. Therefore, the ability of a cultivar to withstand in obtaining acquired variance reflects its genetic background and buffering (Fasoula and Fasoula 1997a, b, 2000, 2002; Tokatlidis et al. 1999).

The coefficient of variation (CV) is the most widely used parameter to quantify phenotypic variation among individual plants in a crop stand. Apart from variation originated from genetic differences, the CV represents environmentally induced variance (Steel et al. 1997); therefore, it can be used to assess stability (Fasoula and Fasoula 1997a, b, 2002; Taylor et al. 1999; Tokatlidis and Koutroubas 2004; Fasoula and Tollenaar 2005). Moreover, the association between load of deleterious genes and inbreeding depression (Fehr 1987) allow using the CV as a relative estimate of the genetic buffering, thanks to its close connection with inbreeding depression (Fasoula and Fasoula 1997a, b, 2000, 2002; Tokatlidis et al. 1999, 2008a).

The load of deleterious genes affects stability, and this was quantified by the CV in the isolation environment in  $F_2$  of two cultivars belonging to crops with a different position in the evolutionary scale (Fasoula and Fasoula 1997a, b). In the vegetatively propagated potato, the single-plant yield distribution was skewed towards the low yield having a high CV of 94%, whilst in the autogamous tomato, a normal yield distribution was obtained with a CV of 26% (Fasoula and Fasoula 1997b). Following gene fixation in potato, unfavourable gene-to-gene interactions on account of the high load of deleterious genes led to unstable performance, whilst in tomato improved gene-to-gene interactions due to the reduced load of deleterious genes resulted in a much better stability. Great load of deleterious genes impair both productivity and stability or buffering, and the impact is reflected by a high CV in the isolation environment; in contrast, the smaller the CV, the more favourable gene-to-gene interactions occur and the more increased productivity and stability are (Fasoulas 1993; Fasoula and Fasoula 1997a, b, 2000, 2002; Fasoula and Tollenaar 2005; Tokatlidis et al. 1999, 2008a).

Informative from this viewpoint are results from Tokatlidis et al. (1999) regarding two contrasting maize materials (Table 1). Inbred lines B73 and Mo17 and their single-cross hybrid as well as two sets of experimental  $S_6$  lines,

**Table 1** Performance in plant yield potential (grams/plant), co-efficient of variation (CV) and mid-parent heterosis of two contrasting maize materials reflecting the load of deleterious genes (data from Tokatlidis et al. 1999)

	B73	Mo17	B73xMo17	A	B	A × B
Grams/plant	75	46	449	281	376	1,113
CV (%)	88	62	38	39	39	30
Heterosis (%)			743			339

named A and B, and their single-cross hybrids were tested in the isolation environment, i.e. under 0.74 plants per square metre. The experimental lines had been derived from the F<sub>2</sub> of the commercial hybrid PR 3183 through continuous selfing and on the basis of line performance per se aiming to exploit additive gene action. Lines B73 and Mo17, devoid of buffering due to the high load of deleterious genes, had low plant yield potential and high CV values. Thanks to good combining ability, their hybrid exhibited 743% higher yield per plant and 50% lower CV than the respective mid-parent values. On the other hand, A and B lines, carrying a lower load of deleterious genes, gave hybrids that exposed 147% higher yield potential than B73xMo17, although exhibiting 54% lower heterosis.

Important implications arise from considering hybrids versus parents based on the above data. Firstly, they constitute a good paradigm of how heterozygosity hides the defective effects of deleterious genes amending the genotype buffering. Secondly, they demonstrate that even mono-genotypic cultivars may show different tolerance to environmentally induced variance, depending on their genetic buffering. Thirdly, they are supportive of an inbreeding strategy as a mean of upgrading the yield potential with parallel elimination of deleterious genes even in a typically cross-pollinated crop. The A × B hybrids developed by this strategy, in comparison with their original hybrid PR 3183, exhibited up to a 115% significantly higher yield potential per plant (Tokatlidis et al. 1998). Their relatively low heterosis value suggests that the improved yield potential was due to homozygote advantage following fixation of favourable additive alleles partly expressed as heterozygotes in PR 3183 (Tokatlidis et al. 1999, 2008a).

It is clear that genetically homogeneous cultivars with good buffering and high yield potential increase resource use efficiency and provide higher mean yields.

### 2.2.3 Stand uniformity and productivity

It is now well known that stand uniformity, determined by plant-to-plant variability, is an essential contributor to the final crop productivity (Gravois and Helms 1996; Taylor et al. 1999; Tollenaar and Wu 1999; Pan et al. 2003; Martin et al. 2005). The highest productivity is achieved when all plants in the crop stand yield the same, that is, when plants share the growth resources equally. Therefore, in uniform

stands, competition is optimized and efficient resource use is achieved, which is a key contributor to optimize productivity (Fasoula and Fasoula 1997a, 2002; Tokatlidis and Koutroubas 2004; Fasoula and Tollenaar 2005).

In a review work, Taylor et al. (1999) analysed data from 362 wheat trials and found that mean yields and the respective CVs exhibited a significant negative correlation. The data showed that as CV increased from 5.5% to 25%, the mean yield declined from 6,000 to 1,000 kg/ha, whilst CV values around 12.5% corresponded to a 50% yield loss. In another work, data from six maize hybrids also depicted a negative relationship between plant-to-plant variability and grain yield per unit area; according to the provided equation, a CV increase from 30% to 60% was accompanied by a 35% yield decrease (Tollenaar and Wu 1999). Tokatlidis and Koutroubas (2004), as well as Fasoula and Tollenaar (2005), presented evidence in maize that productivity optimizes in uniform stands, and contrarily, the larger the interplant differences in growth and development, the stronger the established competition and the less the yield per unit area. This happens because the lower than average yields of some plants are undercompensated by the higher yields of others, implying inefficient resource use.

Since by definition minimal interference among plants excludes the presence of competitive advantages and disadvantages, it is implied that the principal property of a genotype is not the competitive ability but the individual buffering. In turn, it is self-evident that the crop environment is the only choice to optimize resource use efficiency, on the presupposition that the cultivated mono-genotypic cultivar has high yield potential and stability. The importance of mono-genotypic cultivars for stand uniformity and the subsequent benefits are best demonstrated by the replacement of the genetically heterogeneous double-cross maize hybrids by the genetically homogeneous single-cross hybrids. Data from Duvick (1992) showed that the single-cross hybrids were superior over the double-cross hybrids not only in favourable environments but also in lower input agriculture and harsher growing conditions. Initially, single-cross hybrids were deemed less stable than double-cross hybrids, but it is now clear that this conclusion would not have been reached if it was possible to envisage the arrival of the highly buffered single-cross hybrids of the 1970s and later (Fasoula and Fasoula 1997a).

The data provided by Ipsilantis and Vafias (2005) interpret collectively the influence of purity and uniformity

of the stand as well as cultivar buffering on resource use efficiency. Two widely cultivated maize hybrids in Greece were studied across five planting densities. The material included the hybrid alone, i.e.  $F_1$ , the mechanical  $1F_1:1F_2$  mixture and the  $F_2$  alone, whilst the densities were 4.44, 5.33, 6.67, 8.89 and 13.3 plants per square metre. Images in Fig. 2 explicate the following interesting inferences. Stand uniformity is of paramount importance because as plant-to-plant variability expands, the proportion of no-yielding plants enlarges. The impact is stronger in genetically heterogeneous stands due to higher crop variation. For instance, across the two intermediate densities of 6.67 and 8.89 plants per square metre, which define the farming density limits, for the hybrid ‘Rio Grande’, barrenness of 2.1% enlarged to 13% for the mid-heterogeneous  $F_1+F_2$  and to 19% for the high-heterogeneous  $F_2$  (Fig. 2a). Figure 2 also illustrates that the better buffered hybrid ‘Costanza’ suffered less from barrenness. Mean ‘Rio Grande’ CV values of 34%, 55% and 59% for  $F_1$ ,  $F_1+F_2$  and  $F_2$ , respectively, were accompanied by 2.3%, 12% and 15% barrenness, whilst the respective ‘Costanza’ CVs were 30%, 44% and 54% and barrenness values were 1.7%, 4.7% and 10% Fig. 2b.

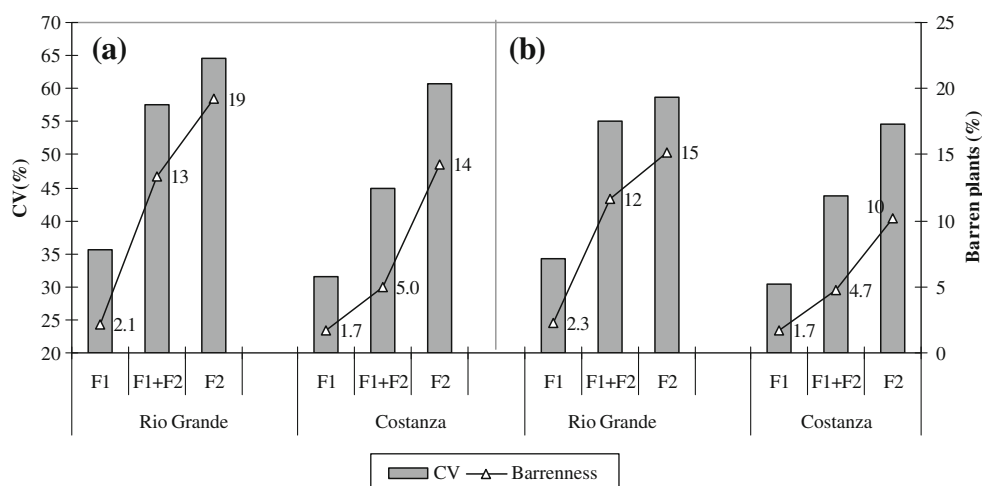
Relevant to the concepts of crop versus competition environment and cultivar’s buffering is the intercropping agricultural system. Several agronomists proposed traditional multispecies systems for designing sustainable cropping systems (Altieri 2002; Malézieux et al. 2009). In this case, crop and cultivar buffering play a determinant role in the overall performance of the intercropping systems.

For example, when common vetch was intercropped with various cereals, i.e. oat, wheat, barley, triticale, the

mixtures were more productive than the monocrop of vetch, but less productive than the cereal sole crop (Caballero et al. 1995; Lithourgidis et al. 2007; Vasilakoglou et al. 2008). Similarly, when a bean landrace was intercropped with two maize hybrids, the bean landrace in pure stand gave 30% less silage yield than the average yield of the two intercrops, but the maize hybrids as sole crops gave 40% and 50% higher silage yield than the respective intercrops (Lithourgidis et al. 2008). In these studies, maize, wheat, barley and oat appeared better adaptable in pure rather than in mixed stands. On the other hand, vetch and bean crops performed better in mixed rather than in pure stands. This implies that the relative contribution of different crops to intercrop performance may be conflicting, attributable to the different genetic background.

If that is the case, then there is a high possibility that breeding may lead to cultivars that would perform better as sole crops rather than in intercrops. This is supported by the finding that in intercropping studies which involve different cultivars of the same crop, a significant genotype by cropping system interaction occurs, demonstrating that intercropping systems favour more genotypes that perform poorly as sole crops rather than the highly performing when grown alone (Davis et al. 1984; Tsay et al. 1988; Santalla et al. 2001; Tefera and Tana 2002; Hauggaard-Nielsen and Jensen 2001; Atuahene-Amankwa et al. 2004; O’Leary and Smith 2004; Gebeyehu et al. 2006).

Genetic buffering of a cultivar is crucial to grade it as suitable for mixed systems. Cultivars that perform poorly in pure stand appear suitable for intercropping. On the other hand, high-yielding cultivars as sole crops will perform



**Fig. 2** Coefficients of variation (CV) for individual plant yields and barren plants of maize hybrid alone ( $F_1$ ), of mixture with its  $F_2$  ( $F_1+F_2$ ) and of the  $F_2$  alone across the densities of 6.67 and 8.89 plants per square metre (a) and across five densities, i.e. 4.44–13.3 plants per square metre (b). The major points are (1) larger CVs are

accompanied by higher barrenness; (2) barrenness is lowest in homogenous  $F_1$ 's, middle in intermediately heterogeneous materials ( $F_1+F_2$ ) and highest in highly heterogeneous  $F_2$ 's; (3) hybrid Costanza is better buffered than Rio Grande, reflected by lower CVs and barrenness (data from Ipsilantis and Vafias 2005)



poorly in mixed stands. The root cause for this discrepancy is the negative relationship of yielding with competitive ability (Fasoula 1990; Reynolds et al. 1994; Thomas et al. 1994; Fasoula and Fasoula 1997a, 2000; Janick 1999; Santalla et al. 2001; Pan et al. 2003). In reality, intercropping systems simulate the conditions of the competition environment, which favour strong competitors against the high yielders. Nevertheless, if such a system is to be accepted as advantageous, it should be superior over sole crops of high-yielding and well-buffered cultivars as well. Sharma and Mehta (1988) evaluated soybean–maize intercrops and reported that the highest productivity was obtained in a sole crop, i.e. 9.27 versus 8.43 g/plant of the top intercrop. Newton and Thomas (1992) studied resistance to mildew in barley mixtures and their components using breeding lines with partial resistance. They found that lines having a lower level of resistance and reduced yield in monoculture showed yield advantage in mixtures. In contrast, lines with a higher level of resistance and increased yield in monoculture showed yield disadvantage in mixtures.

Concisely, competition exerts a detrimental influence on crop productivity. The major reason is the unequal share of resources in the presence of competition, that the growth and the development of individuals are suppressed unevenly (Fasoula and Fasoula 1997a, 2002; Fasoula and Tollenaar 2005). A particular cause also is the competition's connection with decreased harvest index due to inefficient resource use, as was documented in bean and wheat genotypes (Davis et al. 1984; Pan et al. 2003; Gebeyehu et al. 2006). Pan et al. (2003) pointed out that greater plant size hierarchy induced by competition implies a high incidence of growth redundancy which is detrimental to the yield performance of a crop population. Optimal productivity is accomplished with minimal competition in the crop stand, implying that any kind of competitive ability of the individual plants, either acquired or genetic, is undesirable (Fasoula and Fasoula 1997a). The acquired competition can be optimized via agronomic practices, whilst the genetic competitive ability is excluded in monoculture crops. Therefore, crop environment is the only condition to ensure effective use of resources in the farmer's field, and there is a great necessity for well-buffered mono-genotypic cultivars to be developed, suited for the specific conditions of sustainable agriculture.

### 3 The isolation environment optimizes response to selection

The quantitative nature of important agronomic attributes, like productivity, stability, adaptability, etc., constitutes the major obstacle that breeders encounter in their attempt to breed new cultivars. Quantitative traits are vulnerable to

environmental forces responsible for the genotype-to-phenotype gap. For instance, in multi-environmental trials of wheat, pea, soybean and maize, the environment effect has been shown to be preponderant, representing some 50–80% of total variation, whilst variation due to the genotype effect was only 10–25% (Lecomte et al. 2010).

Phenotypic variance ( $s^2$ ) is consummated by three main contributors, the genotypic variance ( $s_g^2$ ), the environmental variance ( $s_e^2$ ) and the variance on account of the genotype by environment interaction ( $s_{g \times e}^2$ ):  $s^2 = s_g^2 + s_e^2 + s_{g \times e}^2$  (Fehr 1987). Falconer (1989) proposed the general equation of expected response to selection ( $R$ ) that involves three determinant elements, i.e. selection intensity ( $i$ ), heritability ( $h^2$ ) and phenotypic standard deviation of the population ( $s$ ):  $R = ih^2s$ . According to the equation, selection effectiveness enlarges by establishing growing conditions that allow to select as few superior plants as possible (high  $i$ ), ensure constant transmission of the phenotypic superiority (large  $h^2$ ) and enhance phenotypic differentiation (large  $s$ ). A large phenotypic variance, however, is meaningful only when the larger possible share belongs to the genetic differences and neither to the environmental influence nor to the genotype by environment variance, so that the heritability maximizes, i.e.  $h^2 = s_g^2 / (s_g^2 + s_e^2 + s_{g \times e}^2)$  (Fasoula and Fasoula 2002; Tokatlidis et al. 2010a).

In breeding research, the plant population considered for selection is genetically heterogeneous, i.e. consists of a mixture of pure lines and/or segregating genotypes. Thus, a breeder has two options, that is, to employ the selection process either in the competition or in the isolation environment. Genes controlling genetic competition are negatively correlated with genes controlling yielding ability. This negative correlation prevents response to selection in the competition environment due to the preferential selection of plants that are strong competitors at the expense of plants that are high yielders (Fasoula and Fasoula 1997a). The existence and measurement of the negative correlation between yielding and competitive ability, as well as its implication on selection efficiency and cultivar degeneration, were studied and elucidated by two landmark papers. One paper was by Kyriakou and Fasoulas (1985) and the other by Fasoula (1990). These papers allow considering the isolation against the competition environment regarding the general equation of expected response to selection and its three components.

#### 3.1 Selection intensity ( $i$ ) in the isolation versus the competition environment

Kyriakou and Fasoulas (1985) used a rye population of more than 4,000 plants and grew half of the plants under competition (15-cm plant spacing) and half in the isolation environment (90-cm plant spacing). In each population,

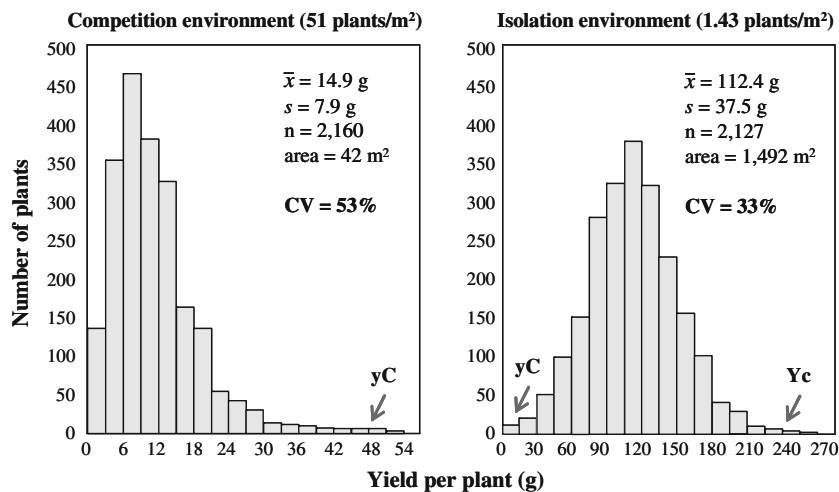
they applied three different selection pressures, i.e. 14.3%, 5.3%, and 1.6%, and the results are presented in Figs. 3 and 4. As Fig. 3 shows, competition converts a normal yield distribution into an L-shaped yield distribution, and it transposes at the right tail end, where selection is realized, the low yielders–strong competitors (yC) instead of the high yielders–weak competitors (Yc). The consequences of the above are shown in Fig. 4 for the three different selection intensities. Response to selection in the rye population increased positively with the increase of selection pressure in the isolation environment, but negatively in the presence of the competition environment. These results provide clear evidence that the competition is the principal factor that prevents efficient selection for yield on a single-plant basis, whereas the absence of competition constitutes the ideal condition for plant breeders to select successfully for high yield.

In the other landmark study on the negative correlation between yielding and competitive ability and the implication on selection efficiency and cultivar degeneration, Fasoula (1990) used foundation seed from an inbreeder, i.e. the soft wheat variety ‘Siete Cerros’, and applied divergent honeycomb selection for high and low yields in more than 2,000 plants. The results provided strong evidence of the high negative correlation between yielding and competitive ability and are presented in Fig. 5. This negative correlation hinders response to selection and is the principal cause of cultivar degeneration. The reason is that constant cultivar reproduction under dense stand as this is commonly practised, which favours the proliferation of low

yielders–strong competitors (yC) at the expense of high yielders–weak competitors (Yc).

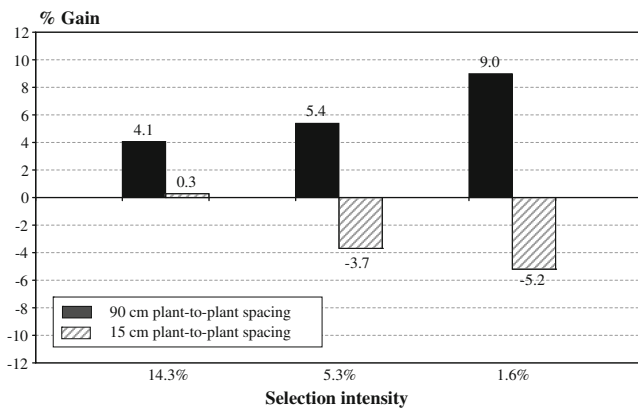
As Fig. 6 shows, line H1 selected in the isolation environment for high-yielding ability outyielded under monoculture the line L3 selected for low-yielding ability by 19%. Conversely, when grown in competition with Siete Cerros, high-yielder line H1 lagged behind low-yielder line L3 by 25%. The implications of these results for plant breeding are crucial. First, they point out that the principal cause of cultivar degeneration is the negative correlation between yielding and competitive ability (Fasoula 1990; Fasoula and Fasoula 1997a; Fasoula and Boerma 2005, 2007; Tokatlidis et al. 2004, 2008b). Most importantly, cultivars of inbreeders, being mixtures of Yc and yC, encompass large amount of exploitable genetic variation and therefore are amenable to continuous improvement. Non-stop selection within cultivars (Fasoula and Fasoula 2000; Fasoula and Boerma 2005, 2007; Tokatlidis et al. 2006, 2011) is also imposed by the constantly released de novo variation through genetic and epigenetic mechanisms, as elegantly discussed by Rasmusson and Phillips (1997) in their classic review and interpretation paper.

These results (Figs. 3, 4, 5 and 6) suggest that because of the negative relationship between competitive and yielding ability, the response to selection for the yield of individual plants is effective in the isolation environment and ineffective in the competition environment. Evidence of the negative correlation between yielding and competitive ability has also been reported in other studies (Reynolds et



**Fig. 3** The competition environment converts a normal yield distribution into an L-shaped distribution. This is because under dense stand, the negative correlation between yielding and competitive ability makes genotypes yC (low yielder–high competitor) to transpose at the right tail end. Conversely, nil-competition in the isolation environment leads to normal yield distribution because of transposition at the right tail end of genotypes Yc (high yielder–low competitor). The general equation of expected response to selection,

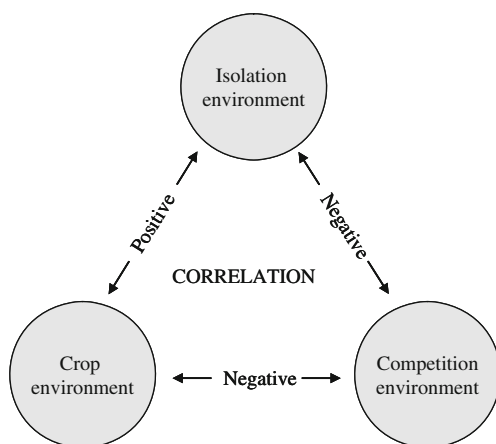
$R = ih^2s$ , is optimized in the isolation environment because: (1) high selection pressures,  $i$ , are applicable without the risk of selecting strong competitors at the expense of high yielders; (2) albeit the additional soil heterogeneity induced by the enlarged experimental area, CV is smaller, implying less acquired variance and higher heritability,  $h^2$ ; and (3) differentiation between plants measured by phenotypic standard deviation,  $s$ , is larger (data from Kyriakou and Fasoulas 1985)



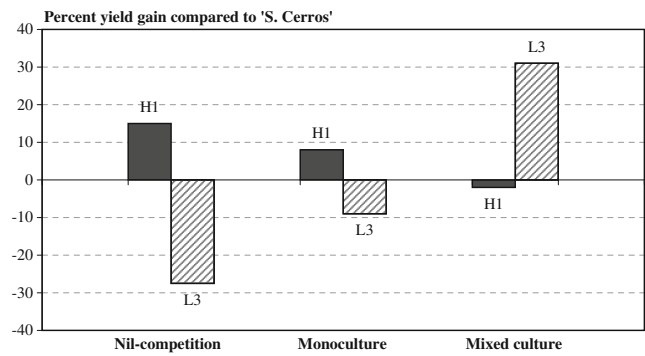
**Fig. 4** Yield response in the rye population of Fig. 3 as affected by the intensity of selection and the plant spacing. Increased selection intensity increases response at ultra-wide plant spacing (90 cm) and reduces response at ultra-narrow plant spacing (15 cm). This testifies that response to selection decreases with the increase of selection intensity under competition and increases only in the absence of competition (data from Kyriakou and Fasoulas 1985)

al. 1994; Thomas et al. 1994; Fasoula and Fasoula 1997a; Janick 1999; Santalla et al. 2001; Gebeyehu et al. 2006). Pan et al. (2003) commented that a competitive ideotype does not maximize reproductive allocation in a population sense and suggested a ‘communal’ ideotype or ‘weak competitor’ to optimize crop productivity.

It is important to notice that the rye population studied by Kyriakou and Fasoulas (1985) was a product of natural selection in the sense that it was reproduced for more than 20 years without selection. This means that low yielders–strong competitors are expected to be highly abundant in the initial population. Like this rye population, populations that are propagated in the long term without artificial selection, e.g. landraces, accumulate genotypes that express



**Fig. 5** Monoculture, i.e. crop environment, of lines selected from ‘Siete Cerros’ is correlated positively with the absence of competition, i.e. isolation environment, ( $r=0.85$ ), and the two are correlated negatively when selected lines are grown in mixed culture with ‘S. Cerros’, i.e. competition environment ( $r=-0.94$  and  $r=-0.86$ , respectively; data from Fasoula 1990)



**Fig. 6** Per cent yield gain of lines selected for high (H1) and low (L3) yield in the nil-competition within the wheat cultivar ‘S. Cerros’. Under monoculture, H1 outyielded L3 by 19%. Conversely, under mixed culture, H1 lagged behind L3 by 25% (data from Fasoula 1990)

the low yielder–strong competitor phenotype. Such landraces, however, serve as an excellent source material to derive outstanding homogeneous cultivars for sustainable agriculture that produce optimally in pure stand, thanks to resource use efficiency. As explained before, this is feasible only through the selection of high-yielding genotypes in the isolation environment, which enables breeders to employ very high selection intensities.

### 3.2 Heritability ( $h^2$ ) in the isolation versus the competition environment

Coefficient of variation is a statistic measurement that quantifies phenotypic variation among individual plants of a crop stand. For a given genotype, any acquired variance is depicted in its CV value, designated by the entry phenotypic standard deviation ( $s$ ) and the entry overall mean  $\bar{x}$  ( $CV = s/\bar{x}$ ). In turn, CV measures the objectivity of the  $\bar{x}$ , and any CV increase under comparable conditions clearly represent respective heritability deterioration (Fasoula and Fasoula 1997a, 2000, 2002; Fasoula and Tollenaar 2005; Tokatlidis et al. 2010a). Data in Fig. 3 demonstrate that in the isolation environment, where interplant interferences are nonexistent, although soil heterogeneity is high due to larger experimental area, i.e. 1,492 versus 42 m<sup>2</sup>, CV was 38% smaller compared with the competition environment. The impact implies that less acquired variance and thus higher heritability was accomplished in the isolation compared with the competition environment.

There is strong evidence that CV values decline when plant density decreases, with regards to grain yield and other traits (Fasoula and Fasoula 1997a, 2002; Tokatlidis and Koutroubas 2004; Fasoula and Tollenaar 2005; Ipsilantis and Vafias 2005; Tokatlidis et al. 2005, 2010a; Maddonni and Otegui 2006). Strong competition at high densities, by enhancing plant-to-plant variability, affects more drastically the CV than the accumulated soil hetero-

genicity at low densities arising from increased occupied land space (Fasoula and Fasoula 1997a, 2002; Tokatlidis et al. 2010a). Therefore, competition reduces the reliability of the means, whilst critical low densities that minimize CV and optimize heritability ensure effective control of environmental influence on genotypic expression and the highest heritable phenotypic variation.

In a recent work aiming to study how density affects CV of grain yield and ear traits, two sets of seven maize hybrids were tested across four densities, the Rom set under rain-fed conditions for 2 years and the Gr set under irrigation (Tokatlidis et al. 2010a). Data from the two top hybrids of each set are presented in Fig. 7, showing that at the low density of 0.74 plants per square metre, maximum yield per plant and minimum CV were accomplished. Particularly for grain yield, the critical low density at which CV reaches the lowest level was found to depend on the genotype, indicating that to optimize heritability, even lower densities are required for high-yielding than for low-yielding genotypes. When in maize breeding genotypes with high yield potential are looked for, the density of 0.74 plants per square metre was recommended since that plant density allowed the request of such genotypes for minimum environmental variance. These results show that the isolation environment is the key condition to improve heritability.

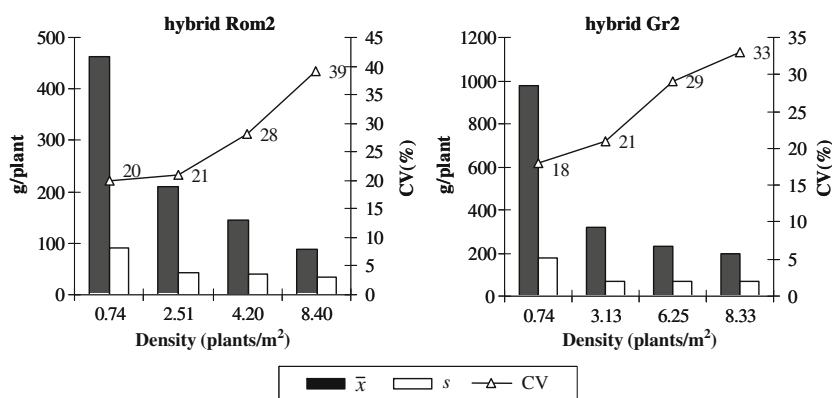
The disturbing effects of the negative association between yielding and competitive ability is a crucial parameter that worsens heritability in the competition environment. This is supported by heritability estimation in intercrops and sole crops. Zimmermann et al. (1984) found larger heritability in sole crops than in intercrops of 16 common bean genotypes with maize for four of the five traits studied, including yield. Sharma and Mehta (1988) estimated in 50 soybean genotypes higher heritability and genetic advance as sole crops than as intercrops with a maize cultivar, considering six traits related to seed yield.

Atuahene-Amankwa et al. (2004) studied the intercropping capacity of 63 bean genotypes with maize and estimated higher heritability in sole crops than intercrops for both seed yield and pod number per plant. These results are supporting the less stressful condition of sole crop to obtain higher heritability than the intercrop situation where stronger competition occurs.

### 3.3 Phenotypic differentiation in the isolation versus the competition environment

Consideration of isolation against competition environment reveals that the maximum phenotypic expression and differentiation is attained in the isolation environment, as Fig. 3 illustrates. Mean yield per plant,  $\bar{x}$ , was almost eightfold higher, and phenotypic standard deviation,  $s$ , was also almost fivefold higher in the absence of competition rather than at the dense stand. Hence, in the isolation environment, higher phenotypic differentiation occurred to satisfy the third part of the general equation of expected response to selection.

Considering Fig. 7, it is also shown that the highest values for both mean yield per plant,  $\bar{x}$ , and phenotypic standard deviation,  $s$ , are reached in the isolation environment. In both hybrids, these measures were respectively about fivefold and 2.7 times higher at the lowest compared with the highest plant density. As a result, about 50% lower CV was obtained at the lowest density. Noticeably, whilst  $\bar{x}$  continuously increases when plant density decreases, changes from the higher to medium densities do not considerably influence  $s$ , found also in other studies (Daynard and Muldoon 1983; Fasoula and Fasoula 2002; Ipsilantis and Vafias 2005). Further plant density decrease will enlarge  $s$ , which maximizes when any plant-to-plant interference is absent. Consequently, the isolation environment is the key to enlarge  $s$  and meet this requirement of the general equation of expected response to selection.

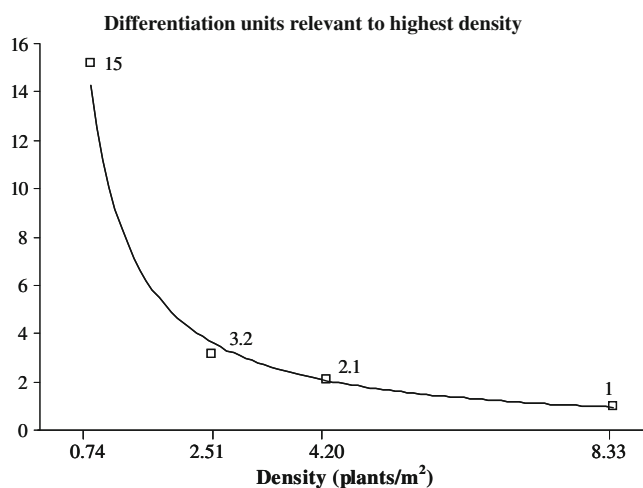


**Fig. 7** Data across four densities, i.e. the lowest proximal to the isolation environment and the highest proximal to the crop density, of hybrid Rom2 grown at rain-fed conditions and hybrid Gr2 grown under irrigation. As density decreases, mean yield per plant ( $\bar{x}$ )

increases at a higher rate than the respective standard deviation ( $s$ ), leading to declining CV values. In the isolation environment, maximum  $\bar{x}$ , maximum  $s$  and minimum CV are accomplished (data from Tokatlidis et al. 2010a)

As phenotypic expression enlarges when plant density decreases, it is usually accompanied by enlarged differences among entries (Fasoulas 1988; Fasoula and Fasoula 1997a, 2000; Traka-Mavrona 1996; Fasoula and Tollenaar 2005; Ipsilantis and Vafias 2005; Maddonni and Otegui 2006). Data from four maize hybrids across a wide range of densities, i.e. 3–12 plants per square metre, showed that the difference in grain yield between the top and bottom hybrids enlarged constantly from 23 g/plant at the highest density to 85 g/plant at the lowest density (Maddonni and Otegui 2006). As Fig. 8 demonstrates, differentiation is maximized when plant density approaches the isolation environment, thus facilitating recognition of the superior genotypes and beneficially contributing to heritability. The isolation environment allowed the identification of even the within-cultivar limited genetic variation and application of effective single plant selection in maize, wheat, soybean and cotton (Fasoula 1990; Fasoulas 2000; Tokatlidis 2000; Fasoula and Boerma 2005, 2007; Tokatlidis et al. 2004, 2006, 2008b, 2011). At high densities, uncontrollable environmental and soil variations can easily turn small differences into non-significant, but this is unlikely to happen with the large differences that occur in the isolation environment (Fasoula and Tollenaar 2005).

In brief, the isolation environment is ideal for effective selection of superior genotypes. Accordingly, the isolation environment allows the application of high selection intensities ( $i$ ), optimizes heritability  $h^2$  and broadens the phenotypic differentiation ( $s$ ), thus fully satisfying the equation for response to selection.



**Fig. 8** Differentiation units across densities relevant to the highest density based on the top–bottom grain yield gap among seven maize hybrids grown under rain-fed conditions. This means that the difference between the highest and the lowest yielding hybrids was by 2.1, 3.2 and 15 times greater at 4.20, 3.2 and 0.74 plants per square metre, respectively, compared with the higher density of 8.33 plants per square metre (adapted from Tokatlidis et al. 2010a)

#### 4 Honeycomb breeding meets entirely the needs of a sustainable agriculture

Sustainability in agriculture is dependent on two things. One is the maintenance and increase of growth resources and the other is the breeding of cultivars exploiting the available resources in the most efficient way. Production environments range according to the available resources from extremely favourable to extremely marginal. In addition, if we also consider the changes due to the climate and cultural practices, the developed cultivars must be constantly modified and improved to meet the new needs. This necessitates that the developed cultivars are regularly selected in order to exploit the constantly released novel variation and to avoid deterioration, as has been explained in previous sections.

Consequently, sustainable agriculture has to be provided with cultivars that meet future challenges. Such cultivars should produce adequately, have improved resource use efficiency, tolerate biotic and abiotic adversities and be capable of adaptation to diversifying environmental conditions. In turn, breeding for sustainable agriculture is necessary to rely on the whole-plant phenotypic evaluation rather than on the single-trait evaluation. A new breeding approach is needed that will render selection more effective and considers the concept of whole-plant evaluation, which recognizes that genes controlling crop yield concern the genome as a whole. Honeycomb breeding is the methodology which emerged as a result of a long-lasting search for the causes limiting efficiency in plant breeding. This endeavour which started 40 years ago (Fasoulas 1988, 1993) was finalized in the following package of five principal causes that affect selection efficiency (Fasoula and Fasoula 1997a, b, 2000, 2002; Fasoula 2008, 2009): (1) density and competition, (2) soil heterogeneity, (3) heterozygosity, (4) genotype by environment ( $G \times E$ ) interaction, (5) unawareness of novel variation.

The study of the effects of density and competition on crop yield and selection efficiency has been discussed extensively in the previous sections and unravelled important findings for plant breeders. The conclusion is that single plant selection for high yield is effective only when it is realized in the absence of competition for growth resources (isolation environment). This brings the following question. How can one relate plant yield potential assessed in the isolation environment with crop yield potential? This question was answered by Fasoula and Fasoula (2000) who partitioned the crop yield potential of an entry into three components, all measured in the absence of competition. The three components are shown in Fig. 9 and are: (1) the entry yield potential measured by the entry mean ( $\bar{x}$ ), (2) the entry stability of

performance measured by the entry standardized mean ( $\bar{x}/s$ ) and (3) the entry adaptability or responsiveness to inputs measured by the standardized selection differential  $[(\bar{x}_{sel} - \bar{x})/s]$ . These three parameters are maximized when plants are grown in the absence of competition and are minimized under dense stand.

Fasoula (2008, 2009) took the product of the three aforementioned components to generate the following two-parameter general response equation

$$R = (\bar{x}_{sel} - \bar{x}) \cdot (\bar{x}/s)^2$$

namely, the product of the selection differential and the stability parameter. The stability parameter,  $(\bar{x}/s)^2$ , was coined the term coefficient of homeostasis (CH). To increase genetic gain through selection using the general response equation, evaluation should be performed in the absence of competition where the two parameters are maximized. Selection differential is maximized if the selected plants are reduced to one. However, reducing the number of selected plants to one is risky because of the confounding effect of soil heterogeneity on single-plant yields, which reduces the selection efficiency. This problem is eliminated by the use of the honeycomb selection designs (Fasoulas and Fasoula 1995) which are advanced experimental designs tailored to maximize selection efficiency by reducing the effect of soil heterogeneity on single-plant yields.

An example of honeycomb selection designs is shown in Fig. 10. The D-31 honeycomb design evaluates 31 genetic entries. The plants of the 31 entries are arranged in the field in horizontal rows in an ascending order, so that planting becomes trouble-free and mistakes are avoided. The only thing requiring attention is the starting number, which differs from row to row and is given by a specific algorithm (Fasoulas and Fasoula 1995). As shown in Fig. 10, every plant occupies the centre of a moving circular complete replicate demonstrated in our example by three plants that belong to entry 24. This symmetric layout permits using the

average yield of the plants included in each moving complete replicate as a common denominator and convert the yield of each plant into a unitless index  $(x/\bar{x}_r)$ , where  $x$  is the yield of each plant and  $\bar{x}_r$  is the mean yield of the plants included in each moving replicate or ring. The plant yield index, squared to increase resolution  $(x/\bar{x}_r)^2$ , is devoid of the masking effect of soil heterogeneity and allows ranking plants efficiently according to their true yield potential (Fasoula 2008, 2009).

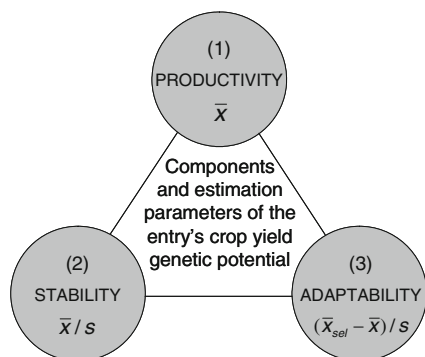
Fasoula (2008, 2009) used the plant yield index (PVI) to replace the selection differential in the general response equation and to produce the following single plant selection equation:

$$\text{Equation A} = (x/\bar{x}_r)^2 \cdot (\bar{x}/s)^2$$

Equation A ensures four things: (1) evaluation of all plants with the same accuracy, (2) conversion of the plant yield potential into crop yield potential, (3) development of density-neutral cultivars, i.e. cultivars that yield optimally over a wider range of plant densities, and (4) application of high selection pressures that maximize genetic gain.

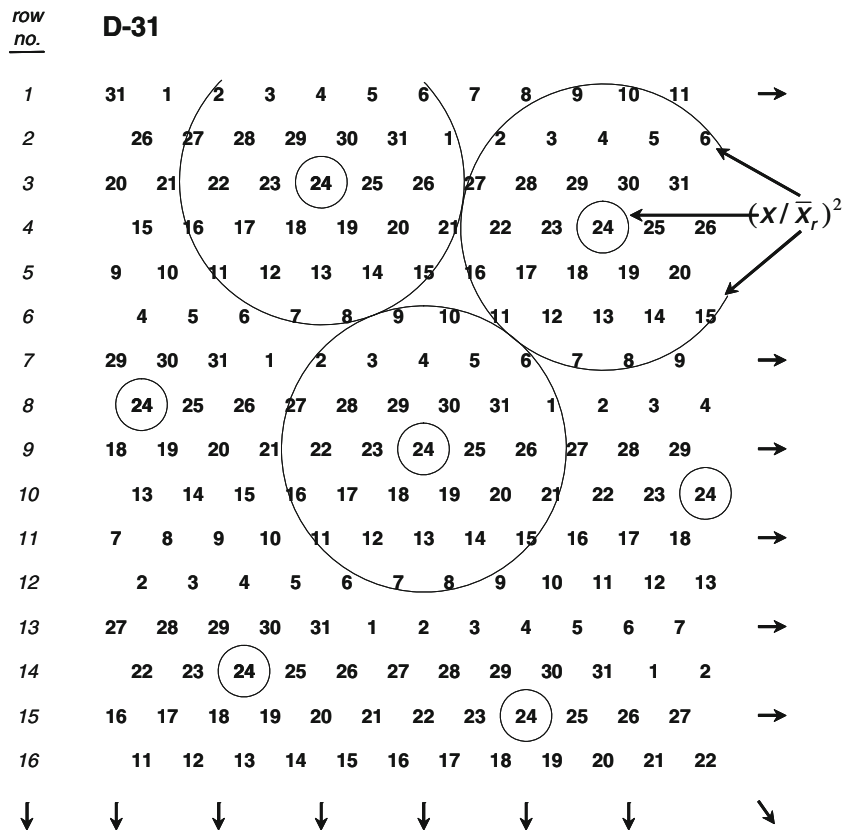
Another version of the D-31 honeycomb design is shown in Fig. 11 illustrating another important feature of the honeycomb selection designs, i.e. even allocation of entries across the field (Fasoulas and Fasoula 1995). In these designs, plants of each entry are allocated in the corners of a triangular grid pattern which covers the whole field. This is exemplified in Fig. 11 for plants that belong to entry 24, and it is accurate for plants of all other entries. The triangular grid pattern, by sampling soil heterogeneity more effectively than random allocation, ensures reliable measurement of the entry coefficient of homeostasis,  $CH = (\bar{x}/s)^2$  and, thus, reliable assessment of the entry stability of performance.

The honeycomb selection designs ensure reliable measurement of the two parameters of Equation A which enables single plant selection for high, stable and density-neutral crop yield. The possible numbers  $N$  capable of forming honeycomb selection designs is given by the formula  $N = X^2 + XY + Y^2$ , where  $X$  and  $Y$  are whole numbers from 0 to infinity. Therefore, the honeycomb designs can accommodate from 3 entries to >250 entries and all have the same unique properties discussed in more detail in Fasoulas and Fasoula (1995). The aforementioned two properties shown in Figs. 10 and 11 are the most important ones because (1) they realize efficient sampling of soil heterogeneity that enables reliable selection for yield potential and (2) they realize even allocation of entries across the field that enables reliable estimation of the CH, the stability coefficient that measures any factor affecting stability of performance. This allows the possibility to

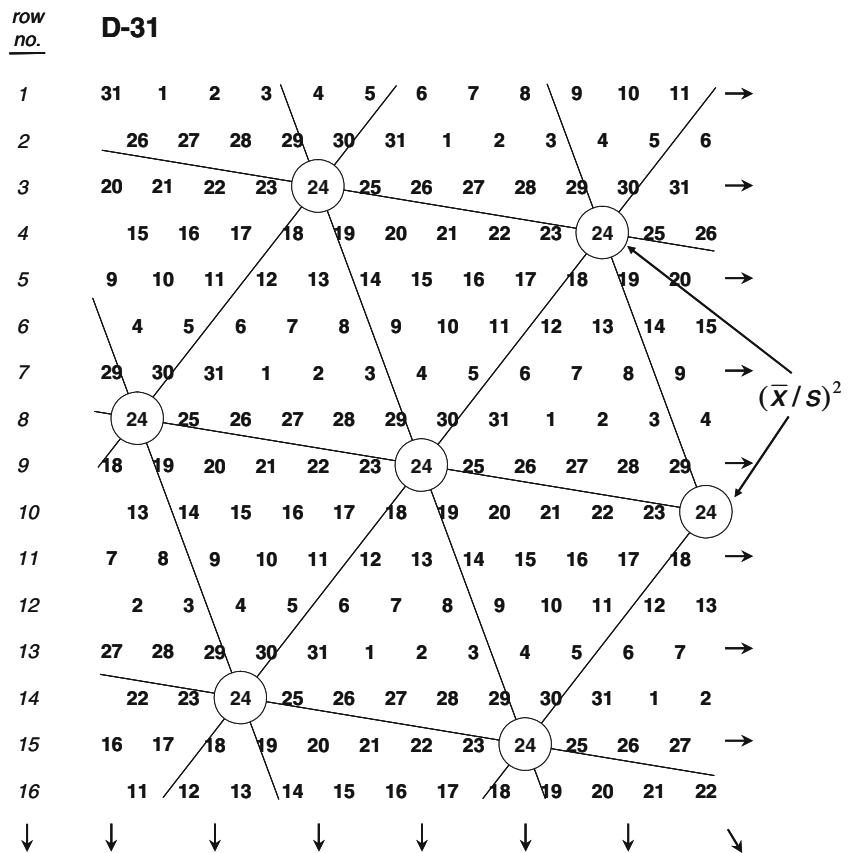


**Fig. 9** The crop yield genetic potential of an entry is controlled by three categories of genes monitoring respectively productivity, adaptability and stability (modified from Fasoula and Fasoula 2000)

**Fig. 10** The honeycomb design D-31 evaluates plants of 31 entries in ascending order and in horizontal field rows. Every plant occupies the centre of a moving circular complete replicate, demonstrated in our example by three plants that belong to entry 24, and its yield is adjusted according to the moving replicate average yield (adapted from Fasoulas and Fasoula 1995)



**Fig. 11** In the honeycomb designs, plants of every entry are allocated in the corners of a triangular grid pattern which covers the whole field and samples effectively soil heterogeneity. The triangular grid pattern is exemplified here for plants that belong to entry 24 (adapted from Fasoulas and Fasoula 1995)



compare entry performance using the following selection equation:

$$\text{Equation } B = (\bar{x}/\bar{x}_t)^2 \cdot (\bar{x}/s)^2$$

where  $\bar{x}$  and  $s$  are the mean yield and standard deviation of each entry and  $\bar{x}_t$  is the trial mean (Fasoula 2008, 2009). Compared with randomized complete block trials (RCB), Equation B enables to evaluate entries more reliably because the first parameter evaluates the yield potential and the second parameter assesses with accuracy entry stability of performance, which is difficult to evaluate in solitary RCB trials.

The formulation of Equations A and B is a step forward in plant breeding because it enables plant breeders to rank objectively plant and entries on the basis of the crop yield potential. This objective ranking allows the application of high selection pressures (1–0.5%) which enhances gene fixation and minimizes the masking effect of heterozygosity on single-plant yields (Fasoula 2008). Furthermore, high selection pressures maximize response through selection and reduce significantly the time required for a cultivar release.

The great advantage of Equation A is that it enables isolating on the basis of the crop yield potential (1) the best entry, (2) the best plants within selected entries, (3) density-neutral plants and (4) plants of high quality. The second parameter of Equations A and B, i.e. the CH, measures in each generation any factor affecting stability of performance, like tolerance to biotic and abiotic stresses including the stress of density, G×E interaction, stand uniformity, epistatic interactions and others. Both equations assess in advance single plant and entry performance for high, stable and density-neutral crop yield; therefore, they require fewer locations and years to accomplish this objective than the traditional methods plant breeding is relying on. In essence, Equations A and B, capitalizing on field experimentation designs that sample and exploit effectively soil heterogeneity, enable selecting in advance for high and stable crop yield even though evaluation is realized in the absence of competition.

Another important advantage of advanced field experimentation designs combined with the two selection Equations A and B is the effective application of non-stop selection within cultivars, local varieties, landraces, populations, synthetic cultivars, etc., in order to constantly improve them for the production environments they are destined to exploit. Non-stop selection by Equations A and B constitutes the key to meeting the requirements of a sustainable agriculture by exploiting the constantly released *de novo* variation in response to various environmental stimuli. This means that if agriculture is to be kept sustainable, developed cultivars must be able to exploit

effectively the constantly changing growth resources on account of changes in climate and cultural practices.

To summarize, evaluation and selection of plants on the basis of Equations A and B maximize genetic gain through accurate whole-plant field evaluation for high, stable and density-neutral crop yield. It therefore serves the needs of a sustainable agriculture by ensuring two important advantages. The first advantage is the large average annual genetic gain. The second advantage is that honeycomb breeding, by developing density-neutral cultivars, exploits effectively not only favourable but marginal environments as well. Marginal environments, having limited resources, are exploited optimally when lower plant populations are used. It is of essence to realize that without the ability of exploiting successfully marginal environments which represent the majority of the production environments globally, sustainability in agriculture becomes problematic.

## 5 Discussion and future perspectives

Intensive agricultural systems are now criticised for human and environmental safety (Malézieux et al. 2009; Rodriguez et al. 2009). The development of sustainable agriculture, characterized by productivity in the long term, adaptability, flexibility, food sufficiency and quality, environmental protection, etc., has been suggested as an alternative system to overcome the problem. Farming systems to be able to address these attributes, however, are heavily reliant on appropriate cultivars. Consequently, the availability of ample supply of cultivars is essential to accomplish the target of sustainable agriculture and food sector and may contribute to agricultural systems less reliant on financial subsidies, with reduced economic fragility and greater recognition of the importance of local self-reliance and production of quality food.

A particular reason for which conventional agriculture has been criticised is the cultivation of genetically homogeneous cultivars (Altieri 2002; Malézieux et al. 2009). However, crop yield is optimized under one condition, that is, when plants are grown in the crop environment, so that preexisting genetic differences do not occur and acquired differences are minimal. Lack of suitable mono-genotypic cultivars may lead to the delusive belief that cultivar homogeneity is the root cause of intensive agriculture inflections. Conversely, appropriate mono-genotypic cultivars may play a key role in stimulating wider adoption of sustainable agriculture and developing of commercially viable farming systems.

Insofar as inbred lines are the uppermost cultivar type, the development of inbred lines adaptable to low-input conditions should be pursued to meet the needs of sustainable agriculture systems. The development of ample



supply of inbred lines for sustainable agriculture offers the possibility of the wider implementation of this practice into the farming systems. First of all, low-yielding capacity will pause to be the major barrier to future growth of sustainable agriculture. Secondly, sustainable agriculture will be a more self-contained system, thanks to the lower cost of certified seed and possibility of farmers to retain their own seed for next season, on the presupposition of isolated propagation in the case of cross-pollinators. The latter is substantial particularly for poor and small-scale farmers and gives the opportunity to improve and enhance rural life because well-adapted cultivars can create the basis for value-added economies in rural areas (Stuber and Hancock 2008).

The masking effects of density and competition on yield response are so decisive that selection for yield on a single-plant basis under competition is not effective. On the contrary, nil-competition optimizes response to selection by allowing the application of high selection pressure, improving heritability and maximizing phenotypic differentiation. Honeycomb methodology, by considering as major principle the application of selection in the isolation environment, counteracts the disturbing effects of competition on selection effectiveness (Fasoula and Fasoula 1997a, b, 2000, 2002). In addition, soil heterogeneity was always considered as an important barrier to employ breeding successfully. The honeycomb experimental designs cope with the confounding implications of soil heterogeneity, thanks to their unique features (Fasoulas and Fasoula 1995). These two important properties of the honeycomb procedure facilitate breeders to consider the individual plant as an evaluating and selection unit that enhances the response to selection and renders the development of improved inbred lines a realizable mission.

To date, sustainable agriculture relies widely on native or locally adapted cultivars, i.e. landraces. These gene pools, being naturally heterogeneous, constitute valuable material for plant breeding to derive new cultivars over the target environments. For instance, for the major cereals, there are many germplasm collections of landraces worldwide exhibiting much variation in valuable agronomic traits, presenting an opportunity to incorporate their positive qualities into new cultivars for more sustainable production through improved deployment and exploitation (Newton et al. 2010). Additionally, in response to environmental changes, endogenous genetic mechanisms broaden the variability of such gene pools (Rasmusson and Phillips 1997; Cullis 2005; Morgante et al. 2005).

Honeycomb breeding has great potential of handling gene pools like heterogeneous landraces or even early released cultivars. This has been validated experimentally in single-plant progeny lines derived from two dry bean landraces that were shown to be tolerant to heat stress and improved in yield

capacity up to 38% (Tokatlidis et al. 2010b), as well as in a lentil landrace under low-input conditions (process is still under way). This method also succeeded in improving biomass yield in *Dactylis glomerata* and *Agropyron cristatum* populations up to 103% and 140%, respectively (Abraham and Fasoulas 2001), and in two switchgrass (*Panicum virgatum* L.) populations up to 25% and 70% (Missaoui et al. 2005). Honeycomb intra-cultivar selection in wheat (Fasoula 1990; Tokatlidis et al. 2004, 2006), maize (Tokatlidis 2000), cotton (Fasoulas 2000; Tokatlidis et al. 2008b, 2011) and soybean (Fasoula and Boerma 2005, 2007) succeeded in upgrading cultivars for yield, stability and important agronomic traits related to product quality and tolerance to stresses. Other reports for successful honeycomb selection exist in cotton, lentil, maize, rice, snap bean and tomato (Traka-Mavrona et al. 2000; Batzios et al. 2001; Ntanos and Roupakias 2001; Tokatlidis et al. 2001, 2005; Christakis and Fasoulas 2002; Vlachostergios et al. 2011). Honeycomb breeding has led to the development and registration of 18 elite soybean germplasm lines with superior agronomic and seed traits (Fasoula et al. 2007a, b, c).

Honeycomb breeding ensures important advantages for the development of cultivars appropriate for sustainable agriculture by considering the following.

*True-to-type cultivars* The kind of pure line cultivar is the ultimate goal of honeycomb breeding (Fasoula and Fasoula 1997b, 2002). By exploiting the additive gene action, pure lines exhibit high yield and stability. Thanks to their homozygous structure, they are self-reproducible and seeds are maintainable at the household level; thus, self-reliance of farmers and local communities increases. This attribute also lowers the cost of certified seed production. Thus, the operating cost of an agricultural system provided with highly buffered pure lines is low.

*Low input* Resource use efficiency is a catalyst to obtain cultivars adaptable to low-input conditions. Resource utilization is optimized when competitive advantages and disadvantages are precluded and inputs are shared equally in the crop. Highly buffered mono-genotypic cultivars bred under low-input conditions have the ability to withstand environmentally induced competition and manage to exploit resources efficiently. Cultivars selected for judicious utilization of outside inputs are environment-friendly, ensuring soil protection in the long run. For organic breeding purposes in lentil, successful employment of honeycomb breeding was reported by Vlachostergios et al. (2011).

*Density-neutral cultivars* Cultivars that are able to use efficiently resources at lower densities and are tolerant to stresses have the capacity to optimize yield at a wider range of different plant densities (Fasoula and Fasoula 2000;

Fasoula and Tollenaar 2005). They are characterized as density-neutral cultivars that contribute to stability of performance particularly for drought-prone and marginal environments. The development of density-neutral cultivars is a realistic target for honeycomb breeding that places particular emphasis on the aforementioned two elements, as was supported by results in maize and wheat (Tokatlidis 2001; Tokatlidis et al. 2001, 2006), and offer important advantages, i.e. lower seeding cost, reduced lodging, ameliorated diseases, alleviated weed and erosion problems (Fasoula and Fasoula 2000, 2002; Tokatlidis and Koutroubas 2004; Tokatlidis et al. 2005; Duvick 2005).

**Biodiversity** A basic rule of sustainable agriculture is food production systems to be rooted in sustaining agricultural biodiversity. The suggested monoculture as an inviolable rule to obtain optimal resource use does not exclude agricultural biodiversity on the condition that many cultivars are grown across the same season. Availability of ample supply of improved cultivars ensures either on-farm or landscape biodiversity and renders the agricultural system both environmentally and economically sustainable.

**Resistance to biotic stresses** Ultra-low plant densities favour canopy infection on the whole because of intensified insect attack or pathogen infection per plant. Thus, honeycomb breeding is very effective in developing cultivars tolerant to biotic stresses. Preliminary data from honeycomb breeding of a lentil landrace opened the possibility of isolating pure line cultivars tolerant to the insect-transmitted and seed-borne bean yellow mosaic virus and alfalfa mosaic virus (Tokatlidis et al., unpublished data). Honeycomb selection within the susceptible cotton cultivar ‘Sindos 80’ was effective in developing two lines tolerant to *Verticillium* wilt (Fasoulas 2000).

**Tolerance to abiotic stresses** Cultivars derived through honeycomb breeding have a usually strong and extensive root system that makes them more resistant to drought as well as to lodging and soil-borne diseases (Fasoula and Fasoula 1997a, 2000). Honeycomb breeding within two climbing dry bean landraces applied either at greenhouse conditions to establish heat stress or at the typical for the crop open field conditions (Tokatlidis et al. 2010b) succeeded in deriving single-plant progeny lines tolerant to heat stress. On the basis of the physiological trait ‘carbon isotope discrimination’ that reflects plant discrimination against  $^{13}\text{CO}_2$  and assimilation of  $^{12}\text{CO}_2$  during photosynthesis, and connected to water use efficiency, possibility of improvement for water use efficiency was validated by honeycomb breeding even within bread wheat and cotton cultivars (Tokatlidis et al. 2004, 2008b).

**Rotation** Crop rotation has great influence on soil N levels, structure and organic matter levels, water and nutrient use efficiency, mycorrhizal associations, grain quality and yield variability, and control of weeds, insects and diseases (Riedell et al. 2009). Provided that farmers have the opportunity to choose among a wide range of cultivars of the crops they alternate, a suitable rotation system can be a profitable practice.

**Weed control** Weeds have by nature strong genetic competitive ability. In turn, a compensatory mechanism for the cultivar reduced genetic competitive ability is indispensable. Such a mechanism is early seed germination, followed by rapid and vigorous plant growth. Cultivars that are developed through honeycomb breeding exhibit early seedling emergence and fast rates of root and shoot growth (Fasoula and Fasoula 1997a). They are characterized by extensive root system and vigorous growth, allowing them to get acquired competitive advantages over weeds.

**Flexibility** Agricultural flexibility, a fundamental factor in achieving sustainability, is secured if plant breeding stays alert continuously and is able to provide producers with new cultivars at all times. The genome responds to climate changes and activates genetic and epigenetic mechanisms that create new genetic variation (Rasmusson and Phillips 1997). Appropriate stewardship of the newly developed variation is an essential contributor to agricultural sustainability in the long run. Plant breeding, therefore, has to be a non-stop process supporting agriculture in perpetuity.

## 6 Conclusions

Competition affects detrimentally resource use efficiency by imposing unequal resource share and uneven suppression of plant growth and development. By inference, either crop performance in the farmer’s field or effectiveness of single plant selection in the breeder’s trial is adversely influenced.

To optimize crop performance in the farmer’s field, two important presupposes have to be met. Firstly, competition in the crop stand should be minimized, implying that the crop environment, i.e. cultivation of mono-genotypic cultivars, is the only condition to ensure effective resource use efficiency. Secondly, well-buffered cultivars are essential to accomplish high productivity and stability, with ‘pure line’ constituting the uppermost cultivar type.

To reduce the genotype-to-phenotype gap and enhance selection effectiveness, the absence of competition, i.e. the isolation environment, constitutes the ideal condition to satisfy the three constituent elements of the general

equation for response to selection. In contrast to the competition environment, the isolation environment allows the application of high selection pressures, optimizes heritability and broadens the phenotypic differentiation.

Honeycomb breeding places particular emphasis on the aforementioned competition influences and has the potential to develop cultivars that serve the needs of a sustainable agriculture. By counteracting the disturbing effects of competition and soil heterogeneity on selection effectiveness, honeycomb methodology has the potential to exploit effectively even marginal environments. The establishment of honeycomb breeding as a non-stop process to deal with the ongoing climate changes and to develop well-adapted ‘pure line’ cultivars with improved productivity can create the basis for value-added economies in rural areas and deliver poverty alleviation and rural development benefits.

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