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ORIGINAL PAPER

Wide- versus specific-adaptation strategy for lucerne breeding in northern Italy

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Abstract This study is aimed at comparing wide- versus specific-adaptation strategies for lucerne in northern Italy on the basis of actual dry matter yield gains over 12 harvests from phenotypic selection, assessing the value of specific genetic bases and selecting environments for the contrasting subregion A (no drought stress/sandy-loam soil) and subregion C (summer drought stress/silty-clay soil). A second aim is to investigate the adaptive responses of five sets of 18 half-sib progenies. The following selected populations were evaluated along with five cultivars: GW–SW, GA–SA, GA–SC, GC–SC and GC–SA (where GW, GA and GC are the genetic bases for wide adaptation, subregions A and C; SW, SA and SC are the selection environments for wide adaptation, subregions A and C). The selection and test environments were four artificial environments created by the factorial combination of two drought stress levels by two soil types. Two environments represented the subregions A and C whereas the combination of the other two environments represented the intermediate subregion B. Genotype × environment interaction ($P \leq 0.001$) due to both environmental factors and implying cross-over interaction between the contrasting subregions occurred for the populations and the five selections. Specific genetic bases (GA and GC) implied gains in their target subregions of 5.2% for subregion A and 2.9% for subregion C compared with the widely adapted one (GW). The gain of SA (‘no stress/sandy-loam soil’) over SC (‘stress/silty-clay soil’) decreased from subregion A (10.6%) through subregion C (1.7%) but exhibited an advantage per se across environments of 5.4%. The best specific selections (GA–SA for subregions A and B; GC–SA for subregion C) implied higher yields of 9.8% in subregion A and 6.5% in subregion C, and over twofold greater selection efficiency across the region, relative to GW–SW. Half-sib progeny × artificial environment interaction ($P \leq 0.05$) occurred in three sets of progenies whose parents belonged to cultivars with different or similar adaptation.

Keywords Adaptation · Breeding strategy · Drought stress · Genotype × environment interaction · *Medicago sativa* · Selection environment

Introduction

Since Turesson’s (1922) pioneering work, a number of studies have revealed specific-adaptation effects for different plant materials and sets of environments. Falconer (1952) pointed out that the yield gain carried over in a target environment from a selection environment is proportional to the genetic correlation between the two environments, suggesting that specific selection environments may be needed for environmentally contrasting cropping areas. The exploitation of repeatable genotype × location (GL) interaction effects by breeding for specific adaptation to distinct, well-defined subregions within a target region has been proposed as an ecological means of raising selection gains relative to breeding for wide adaptation (Bramel-Cox et al. 1991; Ceccarelli 1994; Annicchiarico 2002).

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Breeding programs may produce cultivars with contrasting adaptation patterns by adopting distinct genetic bases (each including material with the desired adaptive response), distinct selection environments (each representative of the target population of environments), or both (Ceccarelli and Grando 1991). Managed or artificial selection environments that possess high genetic correlation with the target environments may be preferred to natural ones for the sake of cost reduction or better control of relevant environmental factors (e.g. drought stress level) (Federer and Scully 1993; Cooper et al. 1995, 1997; Annicchiarico and Mariani 1996). Many breeding programs have investigated GL interaction effects and their implications for breeding, but only some have reportedly compared wide- versus specific-adaptation strategies in terms of actual yield gains, producing indications that are not unequivocal (Atlin and Frey 1990; Singh et al. 1992; Ceccarelli et al. 1998; Annicchiarico et al. 2005).

Previous work (Annicchiarico 1992, 2002; Annicchiarico and Piano 2005) showed that GL interaction effects for dry matter (DM) yield of lucerne cultivars in northern Italy are repeatable in time, imply cross-over interaction of top-ranking material between contrasting locations, relate to specific adaptation to the area where the cultivar or its genetic base originated, and are mainly associated with soil type and the level of summer drought stress of the site. Two geographically defined, contrasting subregions emerged consistently from test site classification, namely: (a) subregion A, placed in the north-western Po valley and characterized by sandy-loam to loam soil and by limited drought stress mainly due to irrigated cropping; (b) subregion C, placed in the south-eastern Po valley and tending to clay soil and severe summer drought due to rainfed cropping and somewhat lower rainfall. Subregion B was intermediate between A and C geographically, for response of cultivars and for environmental characteristics. Four artificial environments created at the breeding site (placed in subregion A) by the factorial combination of soil type (sandy-loam or silty-clay) and drought stress level (almost nil or high) successfully reproduced the adaptive responses across the region of some reference varieties, and confirmed ‘no stress/sandy-loam soil’ and ‘stress/silty-clay soil’ (representing the environments of subregions A and C, respectively) as the most contrasting environments for response of cultivars (Annicchiarico and Piano 2005). The use of these environments represents a cheaper alternative to more selection locations in breeding for wide adaptation to the region or for specific adaptation to each of the two contrasting subregions. The wide variation in adaptive responses of the cultivars suggests an advantage of specific-adaptation over wide-adaptation as a selection strategy, but only the variation in adaptive responses of individual plants and its implications for phenotypic or genotypic selection are really relevant for comparing these strategies in an open-pollinated species such as lucerne.

The main objective of this study was to compare wide- versus specific-adaptation strategies on the basis of actual yield gains from phenotypic selection of lucerne plants performed on the cultivars evaluated in the artificial environments at the end of the experiment reported by Annicchiarico and Piano (2005). For specific adaptation, the study aimed at assessing the effects of: (a) the specific genetic base, assessing the value as germplasm source of populations selected for specific adaptation to the target or the most-contrasting environment; (b) the specific selection environment, assessing the value of plant selection performed in the target or the most-contrasting environment within the same genetic base. A second objective was to investigate the variation in adaptive response among individual plants belonging to the same cultivar or to different cultivars characterized by similar or different adaptation patterns, as revealed by responses of their half-sib progenies (i.e. the most-frequently evaluated material for genotypic selection in lucerne: Rumbaugh et al. 1988).

Materials and methods

Phenotypic selections

The selection of lucerne cultivars that formed the genetic bases for specific- or wide-adaptation strategies, and the phenotypic selection of individual plants in the different selection environments, were performed at the end of the evaluation of 17 populations (13 farm landraces and four varieties) in four artificial environments that is described in Annicchiarico and Piano (2005). The choice of cultivars was based on additive main effects and multiplicative interaction (AMMI)-modeled DM yield over 12 harvests, by which genotype and environment main effects were estimated by analysis of variance (ANOVA) and genotype × environment (GE) effects were estimated by two statistically significant principal component (PC) axes. AMMI modeling is expected to reduce the portion of uncontrolled error variation in the GE interaction (the so-called “noise”) (Gauch 1992). The genetic base to breed for wide adaptation (GW) comprised the three top-yielding populations across environments (landraces ‘8’, ‘17’ and ‘19’), i.e. the top yielding at intermediate environmental conditions (represented by zero value of PC 1 and PC 2 environment scores). While being similar in mean yield, these populations differed for adaptive response. The specific genetic base for subregion A (GA) included the three top-yielding cultivars in the ‘no stress/sandy-loam soil’ environment (landraces ‘17’ and ‘19’ and variety ‘Lodi’). The genetic base for subregion C (GC) comprised the top-yielding material in the ‘stress/silty-clay soil’ environment (landraces ‘3’ and ‘8’ and variety ‘Prosementi’). Phenotypic selection was performed in a stratified manner on the 160 plants that were available for each population in each environment (40 plants per plot harvest area × 4 replications). The plants, grown in density (spaced 7.5 cm between rows and on the row), were ranked according to their DM yield over the fourth and the tenth harvests (which occurred at the end of the stress application in the first and second summer of cropping, respectively) and had to be alive at the end of the testing period (i.e. after three winters). The selection environment for subregion A (SA) was ‘no stress/sandy-loam soil’; that for subregion C (SC) was ‘stress/silty-clay soil’; the environments ‘no stress/silty-clay soil’ and ‘stress/sandy-loam soil’, intermediate for adaptive responses of the cultivars, acted as selection environments for wide adaptation (SW).

Eight plants per population, i.e. 24 individuals overall, were initially selected for each genetic base. They were the two top-yielding plants within each plot for SA and SC environments (selected fraction = 5%), and the top-yielding plant per plot for SW environments (selected fraction = 2.5%). The twofold smaller selected fraction of the widely adapted selection relative to each specifically adapted one, allowed for by the availability of two selection environments instead of one, is consistent with the objective of comparing the two adaptation strategies at similar costs (as implied by the overall adoption of two selection environments also in breeding for specific adaptation). The effect of the selection environment per se was investigated by selecting within each specific genetic base both in the target and the most-contrasting selection environment, producing on the whole the following five selections (named hereafter according to the coded genetic base and selection environment: (a) GW–SW; (b) GA–SA; (c) GA–SC; (d) GC–SA; and (e) GC–SC. The genetic base and the selection environment of these populations are summarized in Table 1. The plants were dug out of the experiment and transplanted as spaced plants in a field in summer 1999. Of the eight individuals per population that initially formed each selection, two were consistently eliminated by spring 2000 for various reasons (mortality, greater susceptibility to viruses or other diseases, etc.) with the aim of maintaining constant the number of parent genotypes per population within each selection. Each final selection included 18 plants (six plants from each of three populations), which were cloned in summer 2000 and polycrossed in isolation cages in late spring 2001 using micro-hives of *Bombus* spp. and ten randomly placed replicated clones per parent to ensure random mating. The high density of pollinators contributed to minimize the rate of inbreeding and its variation among the five selections. Sizeable variation for this characteristic, which may introduce a bias in the comparison of adaptation strategies, was unlikely to occur also on the ground of the similar geographic origin (northern Italy) and the contribution to different genetic bases of the germplasm sources. The seed of each parent in the polycross was harvested separately, obtaining 18 half-sib progenies per population.

Experimental data

The four artificial environments previously used as selection environments acted as test environments to assess the DM yield responses of the five selections and of five cultivars, i.e. the varieties ‘Lodi’, ‘Prosementi’ and ‘Europe’ and the landraces ‘8’ and ‘19’. Four of these cultivars contributed to some genetic bases (Table 1), whereas the varieties of known adaptation pattern across the region acted as reference cultivars (Fox and Rosielle 1982) to verify the ability of the artificial environments to reproduce the subregions in terms of cultivar adaptive response. The environments and their management are described in Annicchiarico and Piano (2005). In brief, they were formed by large (24.0 m × 1.6 m × 0.8 m deep), bottomless containers in concrete laid in a field and filled with local, sandy-loam soil (typical of subregion A) or silty-clay soil imported from subregion C. Irrigated or rainfed cropping during an ordinary summer season were simulated in each environment by irrigation under a moving rain-shelter equipment that covered the crop in rainy days over 12 weeks starting from June 10. During this period, drought-stress environments received a 40 mm irrigation after 4 and 10 weeks, whereas no-stress environments received a 90 mm irrigation after 1, 4, 7 and 10 weeks.

About 6-week old seedlings previously grown in jiffy pots in a glasshouse were transplanted in the artificial environments at the end of October 2001. Within each artificial environment, the ten populations were grown in a randomized complete block design with four replications. Each plot consisted of 144 plants arranged in

Table 1 Genetic base, selection environment and acronym of five lucerne populations selected for wide or specific adaptation to two contrasting subregions A and C

Genetic base[a]		Selection environment[b]	Acronym
Adaptation	Populations		
Subregion A (GA)	Landraces 17 and 19; variety Lodi	Specific for subregion A (SA)	GA–SA
Subregion A (GA)	Landraces 17 and 19; variety Lodi	Specific for subregion C (SC)	GA–SC
Subregion C (GC)	Landraces 3 and 8; variety Prosementi	Specific for subregion C (SC)	GC–SC
Subregion C (GC)	Landraces 3 and 8; variety Prosementi	Specific for subregion A (SA)	GC–SA
Wide adaptation (GW)	Landraces 8, 17 and 19	Intermediate (SW)	GW–SW

For specific adaptation, the adoption of a specific genetic base for the target subregion is associated with selection in the target or the contrasting subregion

[a] Three top-yielding populations across four artificial environments for GW and in the subregion A or C as represented by the environments ‘no drought stress/sandy-loam soil’ and ‘drought stress/silty-clay soil’, respectively, for GA and GC; see Annicchiarico and Piano (2005) for evaluation of populations and geographical definition of subregions

[b] *SA* ‘no drought stress/sandy-loam soil’; *SC* ‘drought stress/silty-clay soil’; intermediate (*SW*) average of ‘drought stress/sandy-loam soil’ and ‘no drought stress/silty-clay soil’ environments

16 rows of 9 plants each, spaced 7.5 cm between rows and 8 cm on the row. The harvest area comprised 72 plants and excluded the four edge rows and three edge plants on the row. The plots of selections comprised a constant number of half-sib progeny plants per parent, i.e. four plants per parent in the harvest area and as many in the border area, randomizing the plants. This arrangement allowed for comparing the five syn-1 selections on the basis of plot yields, and the 18 parents belonging to the same selection on the basis of mean yields of their progeny plants grown in mixture and harvested individually within the plot harvest area.

All environments were fertilized with 40 kg of N, 120 kg of P_2O_5 and 120 kg of K_2O prior to transplanting. DM yield on a plot basis was recorded over six harvests in 2002 and five harvests in 2003. DM yield of individual plants was recorded at the fourth harvest of 2003 and the first and fourth harvests of 2004. The extension of the experiment over part of 2004 was only meant to complete the assessment of parental responses that arise from long-standing growth of their progenies in the artificial environments.

Statistical analysis

Total DM yield over harvests of the 10 populations was submitted to an ANOVA which partitioned the variation into genotype (i.e. population), environment, block within environment, GE interaction and pooled error sources of variation. The variation for environment and GE interaction was partitioned into contrasts relative to soil, drought stress and their interaction. GE effects were also partitioned by AMMI analysis (Gauch 1992), testing the GE interaction PC axes by the F_R test recommended by Piepho (1995). Relationships of genotype × soil and genotype × drought stress interaction effects with the scaled PC scores of cultivars were investigated by simple correlation analysis. Scaled scores of genotypes and environments on the first two PC axes were reported in a biplot.

ANOVA contrasts were also used for assessing the variation among selections and the selection × environment interaction. The variation among selections (four DF) was further partitioned into four linear contrasts that compared the specific genetic bases (GA–SA + GA–SC vs. GC–SA + GC–SC), the specific selection environments (GA–SA + GC–SA vs. GA–SC + GC–SC) and each specific genetic base versus the widely adapted one (GA–SA + GA–SC vs. GW–SW; GC–SA + GC–SC vs. GW–SW), within separate ANOVAs performed for the subregions as represented by the relevant environment(s) (A: ‘no drought stress/sandy-loam soil’; C: ‘drought stress/silty-clay soil’; B: ‘drought stress/sandy-loam soil’ and ‘no drought stress/silty-clay soil’). Other linear contrasts partitioned the selection × environment interaction variation to test the consistency of the differences between genetic bases or selection environments across the contrasting subregions A and C. The best specifically adapted selections and GW–SW were also compared in terms of selection efficiency, i.e. relative yield gain, measuring their gains over the top-yielding control variety in each subregion. Additional linear contrasts were used for comparing these selections and for testing their yield gains for difference to zero (by selection vs. variety yield comparisons). The yield gain over the region (ΔG) provided by different selection strategies was estimated as the weighted mean of the gains in each subregion (as represented by the relevant environment(s)), using as weight the relative crop growing area in each subregion (Annicchiarico 2002):

$$\Delta G = (\Delta G_A P_A) + (\Delta G_B P_B) + (\Delta G_C P_C).$$

Considering the geographical definition of subregions (Annicchiarico 1992; Annicchiarico and Piano 2005) and the available statistics on the crop cultivation (http://www.censagr.istat.it), the relative growing areas of the three subregions were approximately: $P_A = 0.2$; $P_B = 0.4$; and $P_C = 0.4$.

Total DM yield over harvests of each set of half-sib progenies was analysed by ANOVA and AMMI modeling. Nominal yield responses of progenies as a function of the environment PC 1 score were estimated and graphically represented according to Gauch and Zobel (1997). Broad-sense heritability on a family mean basis (h^2) was computed in each environment for each set of half-sib progenies as: $h^2=s_g^2 / (s_g^2+ s_e^2/r)$, where s_g^2 and s_e^2 are the components of variance for variation among progenies and experimental error, respectively, estimated from expectations of ANOVA mean squares, and r is the number of replications.

AMMI analysis was performed by the software IRRISTAT (Version 5), released by the International Rice Research Institute. The Statistical Analysis System (SAS) software was used for all remaining analyses.

Results

Selected populations and cultivars

The environments differed for mean yield, owing to significant effects ($P \le 0.01$) of no drought stress versus stress (+24%) and silty-clay versus sandy-loam soil (+16%). Therefore, 'no stress/silty-clay soil' (33.74 t/ha) and 'stress/sandy-loam soil' (23.23 t/ha) were the top- and bottom-yielding environments, respectively. All selections except GC–SC outyielded any other population for mean yield across environments ($P \le 0.05$) (Table 2). The average yield gain of each selection over the cultivars contributing to its genetic base ranged from 5% for GC–SC to over 20% for GA–SA. 'Europe' was the lowest-yielding entry (Table 2).

Table 2 Mean dry matter yield across four artificial environments of ten lucerne populations

Population	Yield (t/ha)
GA–SA	32.75 a
GA–SC	31.36 ab
GW–SW	30.87 ab
GC–SA	30.26 b
GC–SC	28.42 c
Landrace 8	27.80 c
Lodi	27.33 c
Landrace 19	26.77 c
Prosementi	26.26 c
Europe	21.69 d

Populations with same letter do not differ at $P \le 0.05$ according to Newman–Keuls test

Genotype × drought stress (GD) ($P \le 0.01$), genotype × soil type (GS) ($P \le 0.01$) and genotype × stress × soil interaction ($P \le 0.05$) contributed to GE interaction ($P \le 0.001$) of the ten populations. In the AMMI analysis, PC 1 accounted for 74% and PC 2 for 18% of the GE interaction variation; the latter PC axis being significant at $P \le 0.05$. The environment ordination in the space of these PC axes confirmed 'no stress/sandy-loam soil' and 'stress/silty-clay soil' (representing the contrasting subregions A and C, respectively) as the most contrasting environments for adaptive responses of the entries (Fig. 1), despite their only moderate difference in mean yield (less than 2 t/ha). The ordination on PC 1 was more related to soil type than to drought stress level (as indicated by the similarity of the two environments with silty-clay soil), whereas that on PC 2 represented mainly a contrast of stress versus no-stress environments (Fig. 1). Accordingly, genotype PC 1 scores were more associated with GS interaction effects (defined as positive for better response to silty-clay soil; $r = 0.96$, $P \le 0.01$) than with GD interaction effects (positive for better response to drought; $r = 0.61$, $P \le 0.06$) of the populations, whereas genotype PC 2 scores were correlated with GD interaction effects ($r = 0.79$, $P \le 0.01$).

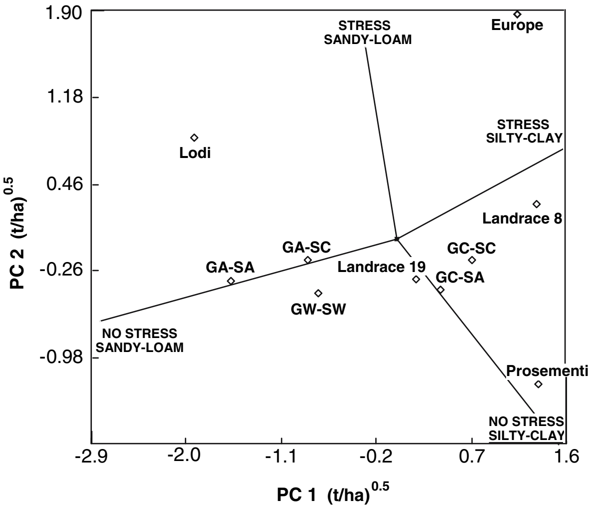

Fig. 1 Scores in the space of the first two genotype × environment interaction principal component (*PC*) axis for dry matter yield of ten lucerne populations and four artificial environments (indicated by *diamonds* and *vectors*, respectively). Acronym of selections indicates the genetic base (GA, GC and GW are for subregion A, subregion C and wide adaptation, respectively) and the selection environment (SA, SC and SW are for subregion A, subregion C and wide adaptation, respectively); the environments 'no drought stress/sandy-loam soil' and 'drought stress/silty-clay soil' are representative of subregions A and C, respectively

The AMMI-modeled GE interaction effect for each genotype–environment combination may be estimated from the biplot in Fig. 1 by summing up the product of the genotype and environment score on each PC axis. Thus, genotype–environment combinations that are close to each other and distant from the origin of PC axes are characterized by large positive GE effects. The adoption of a specific genetic base implied a positive GE effect in the target subregion, as shown by the selections GA–SA and GA–SC in the 'no stress/sandy-loam soil' environment and, although to a lesser extent, by the selections GC–SC and GC–SA in the 'stress/silty-clay soil' environment. The selection environment had a smaller but sizeable effect on the adaptive response, as indicated by the smaller positive GE effects of GA–SC relative to GA–SA in the 'no stress/sandy-loam soil' environment and of GC–SA relative to GC–SC in the 'stress/silty-clay soil' environment. The selection for wide adaptation (GW–SW) showed a slight trend towards positive interaction with 'no stress/sandy-loam soil' (Fig. 1).

'Lodi', contributing to the genetic base for subregion A, was the top-yielding variety in the environment representing this subregion owing to a positive GE effect that arose from specific adaptation to sandy-loam soil (Fig. 1). 'Prosementi', which contributed to the genetic base for subregion C while possessing fairly wide adaptation (Annicchiarico and Piano 2005), responded positively to the 'no stress/silty-clay soil' environment (Fig. 1) and was the top-yielding variety across the environments representing the subregions B and C. 'Europe' confirmed its known (Annicchiarico and Piano 2005) better response to drought stress conditions (Fig. 1). Landrace '19', contributing to the genetic bases for subregion A and for wide adaptation, responded somewhat better to non-stress conditions. Landrace '8', contributing to the genetic bases for subregion C and for wide adaptation, confirmed its better response to the environment representing subregion C (Fig. 1).

Significant ($P \leq 0.001$) interaction between selections and environments took place as a consequence of genotype × soil type ($P \leq 0.01$), genotype × drought stress ($P \leq 0.10$) and genotype × stress × soil ($P \leq 0.10$) interactions. The variation among selections was significant at $P \leq 0.01$ in the environment representing subregion A and across those representing subregion B, and at $P \leq 0.10$ in the environment representing subregion C. Yield values of the selections in each subregion are reported in Table 3.

The specific genetic bases (averaged across selection environments) tended to specific adaptation to their target subregion, as implied by the occurrence ($P \leq 0.01$) and the sign of their interaction with the environments reproducing the subregions A and C (Table 3). Compared with the genetic base for subregion C, that for subregion A exhibited 24.8% higher yield in the conditions of subregion A, 5.9% lower yield in those of subregion C, and higher mean yield across environments that led to better response in the conditions of subregion B and to lack of statistical difference between genetic bases in the environment representing subregion C (Table 3). The specific selection environments (averaged across genetic bases) tended to interact with the contrasting subregions ($P \leq 0.10$), producing material with a relatively better response to its selection conditions (Table 3). This response was more pronounced for the genetic base for subregion A (Table 3). The average advantage as selection environment of 'no stress/sandy-loam soil' over 'stress/silty-clay soil' decreased from subregion A (34.06 t/ha vs. 30.80 t/ha = +10.6%) through subregion C (28.83 t/ha vs. 28.35 t/ha = +1.7%) but was not reversed in the latter subregion by the effect of selection in the target environment (Table 3), owing to an advantage per se across environments equal to +5.4% if estimated as the average advantage of GA–SA + GC–SA over GA–SC + GC–SC across the four environments (31.51 t/ha vs. 29.89 t/ha). On the whole, the specific selections of greatest interest were GA–SA for subregions A and B and GC–SA for subregion C (Table 3). They showed significant cross-over interaction, since GA–SA outyielded GC–SA in the conditions of subregions A (+23.1%, $P \leq 0.01$) and B (+10.1%, $P \leq 0.05$) and were outyielded by GC–SA in those of subregion C (–11.7%, $P \leq 0.05$).

In comparison with the widely adapted genetic base, each specific genetic base (averaged across selection environments) tended to higher yield in the environment representing its target subregion and to lower yield in that representing the most-contrasting subregion, as a consequence of genetic base × environment interactions (Table 3). The advantage of the specific germplasm over GW–SW was larger for subregion A (36.02 t/ha vs. 34.24 t/ha = +5.2%) than for subregion C (29.41 t/ha vs. 28.57 t/ha = +2.9%), but did not reach significance in either case (Table 3). The advantage of the best specific selections over GW–SW was larger, i.e. +9.8% ($P \leq 0.05$) for GA–SA in subregion A and +6.5% ($P > 0.10$) for GC–SA in subregion C (Table 3). GA–SA outyielded GW–SW (+9.0%, $P \leq 0.05$) also in the conditions of subregion B (Table 3).

The yield gains of the best specific selections and the widely adapted selection over the top-yielding variety are reported in Table 4. The gain, i.e. the yield difference between selection and variety, was not signifi-

Table 3 Dry matter yield in three subregions (A–C) as represented by artificial environments for lucerne populations selected for wide or specific adaptation to the most contrasting subregions (A and C), and significance of the interaction between the relevant germplasm effect (genetic base or selection environment) and A or C subregion

Genetic base[a]	Selection environment[a]	Acronym of selection	Yield (t/ha)[b]			Germplasm × (A vs. C) interaction[b]
			A	B	C	
Specific for A (GA)	Specific for A (SA)	GA–SA	37.59	33.09	27.24	–
	Specific for C (SC)	GA–SC	34.44	31.34	28.32	–
	Mean	–	36.02	32.21	27.78	–
Specific for C (GC)	Specific for A (SA)	GC–SA	30.54	30.05	30.43	–
	Specific for C (SC)	GC–SC	27.16	29.07	28.38	–
	Mean	–	28.85	29.56	29.41	–
(GA-GC) mean difference	–	–	+7.17**	+2.65*	–1.63 NS	**
–	(SA-SC) mean difference	–	+3.26*	+1.36 NS	+0.48 NS	^{x}
For wide adaptation (GW)	Intermediate (SW)	GW–SW	34.24	30.34	28.57	–
(GA-GW) mean difference	–	–	+1.78 NS	+1.87 NS	–0.79 NS	^{x}
(GC-GW) mean difference	–	–	–5.39**	–0.78 NS	+0.84 NS	**

[a] Subregions are represented by artificial environments as follows–A: ‘no drought stress/sandy-loam soil’; C: ‘drought stress/silty-clay soil’; B: average of ‘drought stress/sandy-loam soil’ and ‘no drought stress/silty-clay soil’

[b] *NS* not significant

[x] Significant at $P \le 0.10$

[*] Significant at $P \le 0.05$

[**] Significant at $P \le 0.01$

Table 4 Comparison of the best specific selection with the widely adapted one and with the best specific selection for subregion A, based on dry matter yield gain (t/ha) over the top-yielding control variety in three subregions (A–C) and over the region

Comparison	Subregion[a,b]			Region[c]
	A	B	C	
1. Best specific selection	4.12*	6.09*	3.64 NS	4.72
Widely adapted selection	*0.77*	3.34	*1.78*	2.20
Ratio	5.35	1.82	2.04	2.14
2. Best specific selection	4.12	6.09	3.64*	4.72
Best specific selection for A	4.12	6.09	*0.45*	3.44
Ratio	1.00	1.00	8.09	1.37

[a] See Table 2 for definition of subregions and yields of selections. Specific selections are GA–SA (genetic base and selection environment for A) for A and B, and GA–SC (genetic base for C and selection environment for A) for C; control varieties are ‘Lodi’ for A (33.47 t/ha), and ‘Prosementi’ for B (27.00 t/ha) and C (26.79 t/ha)

[b] *NS* selections not different; gains in *italics* are not different from zero at $P \le 0.10$ (following the relevant selection versus variety yield comparison)

[c] Weighted mean across subregions, using as weight the approximate crop growing area in each subregion

[*] Selections different at $P \le 0.05$

cant for GW–SW in subregions A and C. In comparison with GW–SW, the best specific selections provided: (a) over fivefold greater yield gain for subregion A; (b) twofold greater gain for subregion C (despite the lack of statistical difference between selections); (c) about 80% greater gain also for subregion B (where the larger gains of all selections were due to relatively worse adaptation of the varieties); and (d) over twofold greater gain over the region (4.72 t/ha vs. 2.20 t/ha) (Table 4). These results suggested the greater efficiency of a specific-adaptation strategy in which the genetic base is selected specifically for each of the contrasting subregions A and C, the phenotypic selection is performed for both subregions in the environment representing subregion A, and the yield improvement for subregion B relies on carry-over effects of material selected for subregion A. This strategy was compared with another less expensive strategy suggested by the results, namely the specific selection only for subregion A that exploits carry-over effects for subregions B and C. This strategy, however, provided non-significant and eightfold lower yield gain for subregion C, and distinctly lower yield gain over the region (3.44 t/ha vs. 4.72 t/ha), in comparison with that adopting a specific genetic base for subregion C (Table 4).

Half-sib progenies

The separate ANOVAs for the five sets of progenies revealed significant ($P \le 0.05$) progeny × environment interaction for the set whose parents derived from populations with different adaptation (GW–SW) and two sets originated by populations with similar adaptation, i.e. GA–SC and GC–SA. Progeny × soil type ($P \le 0.05$) and progeny × drought stress interactions

($P \leq 0.10$) in the three sets, and progeny × drought × soil interaction ($P \leq 0.10$) in GA–SC and GC–SA sets contributed to GE interaction. In the AMMI analysis of these sets of progenies, PC 1 explained at least 55% of the GE interaction variation (Table 5) and was the only PC significant at $P \leq 0.05$. The environment ordination on PC 1 was very similar to that for populations for the GW–SW set (Table 5; Fig. 1), confirming the contrast between the environments that represented the subregions A and C. For the sets originated by populations with similar adaptation, PC 1 tended to represent a contrast between non-stressed environments with different soil (Table 5).

Nominal yield responses are reported in Fig. 2a for the progenies derived from populations with different adaptation, which are of special interest for appreciating the level of inter- and intra-population variation for adaptive response within the germplasm pool of local interest for breeding. The occurrence of some negative nominal yield was due to subtraction of the main effect of the high-yielding 'no stress/sandy-loam soil' environment (recalling that nominal yields are AMMI-modeled yields from which the environment main effect is eliminated (Gauch and Zobel 1997)). The mean response of the progenies of parents belonging to the same population (Fig. 2b) kept trace of the adaptive response of the source population, reproducing the responses of the landraces '19', '17' and '8' across the four environments that were observed in a previous study (Annicchiarico and Piano 2005). However, the results suggested the occurrence of variation in adaptive response also within landraces (Fig. 2a), in agreement with the occurrence of GE interaction also in two sets of progenies derived from populations with similar adaptation. There was cross-over interaction of top-yielding material between the environments reproducing subregions A and C (Fig. 2a). For example, only two progenies were among the four top-ranking in both environments.

The average broad-sense heritability on a progeny mean basis obtained by averaging the h^2 values relative to the five sets of progenies was 0.56 for 'no stress/sandy-loam soil' and 'stress/sandy-loam soil', 0.53 for 'no stress/silty-clay soil', and 0.46 for 'stress/silty-clay soil'. These values suggested a trend towards higher selection efficiency of the selection environment for subregion A relative to that for subregion C (when applied to the same germplasm pool).

Discussion

GE interaction effects tend to be modest in crops characterized by high levels of heterozygosity and heterogeneity (Becker and Léon 1988; Brancourt-Hulmel et al. 1997). However, large GE effects have often been reported in open-pollinated forage crops (e.g. Ravel and Charmet 1996; Casler et al. 2003), probably owing to the long-standing, specific selection pressures that acted on natural populations or landraces and the large use of such material for variety selection. The wide intra-population variation of Italian landraces and most varieties of lucerne (Annicchiarico 2006) justifies the notable yield gains of the phenotypic selections over their source populations. The reduction of these gains across generations following the Syn-1 is expected to be modest in synthetic varieties of autotetraploids derived from 18 unrelated parents (Busbice and Gurgis 1976), as in this case. The results for half-sib progenies revealed some degree of intra-population variation also for adaptive response, which could be exploited for specific selection. This variation was the main responsible for the somewhat better response to the selection environment than to the most-contrasting environment which emerged from selection within each specific genetic base, given the limited inter-population variation for adaptive response within these sets of populations. However, the GE interaction effects due to the effect of the selection environment were smaller than those due to selection of a specific genetic base, highlighting the importance of evaluating and exploiting the inter-population variation for adaptive response.

The previous assessment of cultivar adaptation in the artificial environments (Annicchiarico and Piano 2005) suggested a closer relationship of GE effects with

Table 5 Genotype × environment (*GE*) interaction variation accounted for, and score of four artificial environments, for the first GE interaction principal component axis in three sets of 18 lucerne half-sib progenies

Progeny set	GE variation (%)	No drought stress/ sandy-loam soil	Drought stress/ sandy-loam soil	No drought stress/ silty-clay soil	Drought stress/ silty-clay soil
GA–SC	57	–3.01	0.35	1.85	0.81
GC–SA	55	–2.51	–0.04	2.07	0.48
GW–SW	70	–3.30	0.44	1.16	1.70

Progeny × environment interaction always significant at $P \leq 0.05$

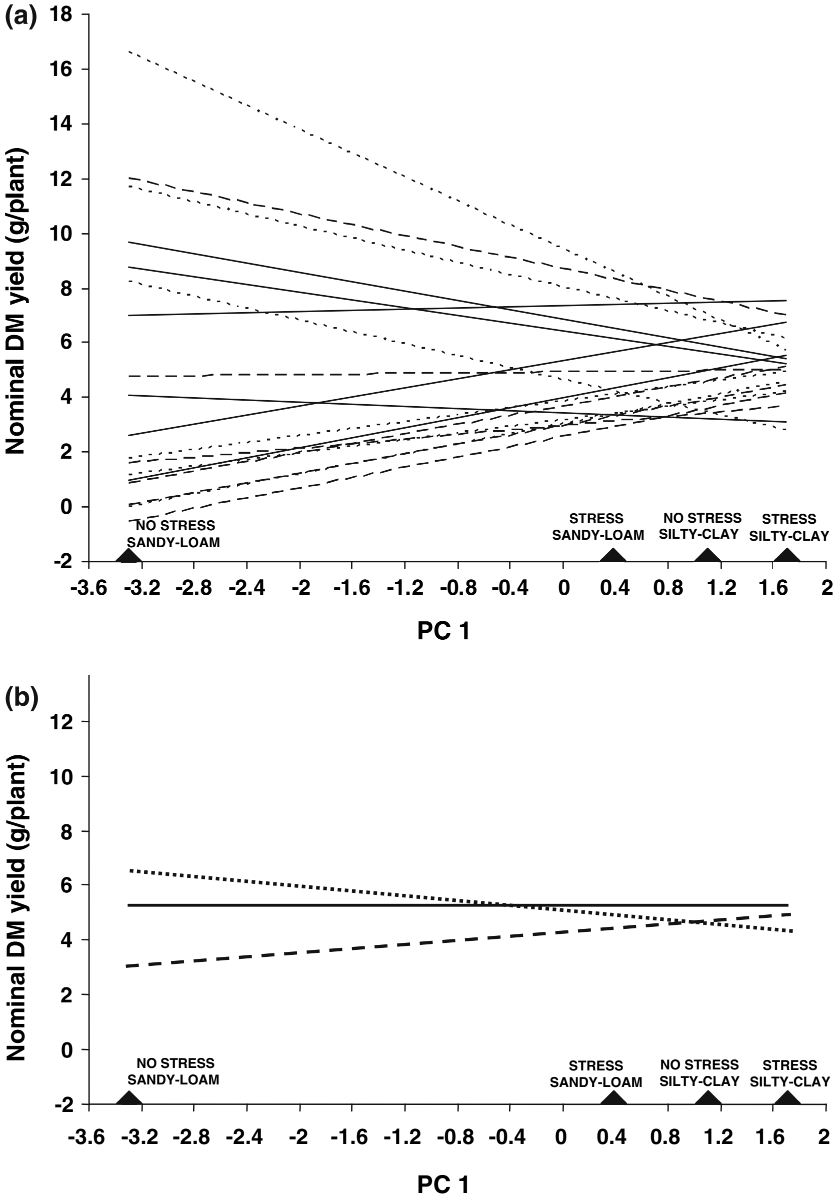

Fig. 2 Nominal dry matter (*DM*) yield of 18 lucerne half-sib progenies as a function of the first genotype × environment interaction principal component (*PC*) axis of artificial environments (**a**), and mean nominal yield of the half-sib progenies of parents belonging to the same population (**b**) (*dotted line* parent genotype from landrace ‘19’; *continuous line* parent genotype from landrace ‘17’; *broken line* parent genotype from landrace ‘8’)

the drought stress factor than with soil type, and a lower similarity between stress and non-stress environments having silty-clay soil. These differences to the present findings may be attributed to the partly different germplasm under study and to the milder drought stress in the first summer that occurred here as a consequence of exceptional rainfall over the 18 days preceding the stress application (133 mm, sufficient to compensate the local Potential Evapo-transpiration demand for almost 5 weeks). Indeed, the average effect of no stress versus stress on total DM yield over the crop cycle was distinctly lower here (+24%) than in the previous study (+31%). An earlier control of water amounts in late spring (by irrigation and the rain-shelter) would be necessary to increase the reproducibility of the drought stress level occurring in subregion C. Another difference between the two studies was the currently lower mean yield of ‘Europe’, possibly due to genetic differences between the two seed lots of this old variety that were used. This study and the previous one (Annicchiarico and Piano 2005) agree on several aspects, including the sharp contrast between the two environments that represent the subregions A and C and the adaptive responses of the cultivars. Those of ‘Lodi’ and ‘Prosementi’ also agree with the known responses of these reference varieties across agricultural environments of the target region (Annicchiarico and Piano 2005).

The somewhat milder drought imposed on the test environments in comparison with the selection environments may have led to some overestimation of the carry-over effects in subregions B and C of specific breeding for A and to some underestimation of the gains obtainable by specific breeding for subregion C. However, the yield advantage of the best specific selection over the widely adapted one was sizable not only for subregion A but also for subregion C when expressed in terms of selection efficiency, suggesting the selection of specific genetic bases for subregions A and C in the contrasting environments and the phenotypic selection for both subregions in the 'no stress/ sandy-loam soil' environment. The specific selection for subregion A targeted to the whole region also showed some interest, although the modest yield gain for subregion C does not encourage its adoption.

The higher selection efficiency for subregion C of the non-target environment over the target one was just a trend in the relevant genetic base and was not significant across genetic bases (Table 3). This trend was determined by an advantage per se of 'no stress/ sandy-loam soil' over 'stress/silty-clay soil' as a selection environment, which may be related to higher narrow-sense heritability for phenotypic selection of individual plants. This hypothesis could not be verified, but agrees with the trend towards higher broad-sense heritability of the former environment relative to the latter that emerged in the evaluation of half-sib progenies. Stressed environments may show lower broad-sense heritability than non-stressed ones (e.g. Atlin and Frey 1990; Ud-Din et al. 1992), but the results vary widely depending on the set of environments (Ceccarelli 1994). The lower heritability for the combination of drought stress and silty-clay soil suggested by the results may partly arise from greater experimental error caused by extensive cracking of the clayish soil under drought. This disadvantage may be large in the phenotypic selection of individual plants but is expected to decrease in genotypic selection schemes, especially when the estimation of the parent value is based on several replications. Indeed, the fairly modest difference in h^2 values observed between the contrasting environments in the half-sib progeny testing suggests that the advantage per se of selecting in the conditions of subregion A may decrease substantially for a genotypic-selection scenario. Assessing more precisely the genotype value in the 'stress/silty-clay soil' environment may also increase the ability to exploit the intra-population variation for adaptive response, further increasing the efficiency of selection for subregion C in the target environment relative to the non-target one. Progeny testing may also allow for another approach to error reduction for specific selection, namely the progeny assessment in the two contrasting environments based on AMMI-modeled yields across the four environments (as in Fig. 2a) rather than on original data. While being valuable for multi-environment noisy data on a theoretical ground (Gauch 1992) and according to empirical evidence (Annicchiarico et al. 2006), this approach would require the use of the four environments instead of the two contrasting ones that are needed for specific selection based on original data.

In conclusion, this study indicates some advantages of selecting distinct genetic bases formed by specifically adapted populations for two subregions of northern Italy, as well as the distinct advantage of selecting in the target environment for one of the two subregions. Despite the simple selection procedure used for its constitution, the selection GA–SA has been promoted for registration in the Italian Register of Varieties (under the name 'Costanza') following its good performance across locations placed in subregions A and B in the official testing trials, thereby confirming in agricultural environments the agronomic value emerging under artificial environments. The yield advantage of 5% over GW–SW (coded as 'MSI001') and of 9% over GA–SA ('Costanza') recently showed by GC–SC (coded as 'MSI004') in an agricultural environment of central Italy characterized by summer drought stress and a silty-clay–loam soil (Torricelli 2006) confirms the interest of specific breeding for conditions of subregion C and suggests that this subregion may extend beyond the south-eastern part of northern Italy. Further work is required to complete the indications on selection environments for this subregion and to verify whether the indications that apply to the current selection scenario (phenotypic selection of individual plants) are also valid in the context of genotypic selection. Information is also lacking on the pattern of genetic gain that may be produced by each adaptation strategy over more than one selection cycle, i.e. in the context of a recurrent selection scheme.

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