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Mixing ability of conventionally bred common vetch (*Vicia sativa* L.) cultivars for grain yield under low-input cultivation

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Abstract

An alternative way to overcome the negative environmental fluctuations observed in low-input culture systems is to compose and utilize cultivar mixtures. However, the available genetic materials to compose such mixtures are cultivars developed by conventional breeding programs with questionable adaptability under low-inputs. The aim of this work was to investigate the mixing ability of conventionally bred common vetch cultivars for grain yield under low-input cultivation. Six common vetch cultivar mixtures were evaluated over their conventionally bred cultivar components for grain yield under low-input cultivation in four environments (2007-2009). Grain yield and stability performance were assessed for each entry. Mixture effect was calculated as an index for the quantitative relation between the mixture and its conventionally bred cultivar components. ANOVA and GGE-biplot analysis indicated that four out of six mixtures over-yielded the average of the experiment and the most cultivar pure stands. Two of the mixtures illustrated high yield, stability across environments and positive Mixture Effect in three out of four environments and could be recommended for low-input cultivation. Earliness and temporal maturing of common vetch cultivar components were recognized as major factors affecting mixture's grain yield and stability performance and should be taken into account when composing common vetch cultivar mixtures for grain yield.

Keywords: Cultivar Mixtures, Low-input Agriculture, Mixing ability, Mixing Advantage, *Vicia sativa* L.
Abbreviations: ANOVA: analysis of variance; Clt: cultivar; Envt: environment; GGE: genotype main effect plus genotype X environment interaction; ME: mixture effect; Mix: mixture; SS_{TRMT}: sum squares treatment.

Introduction

Common vetch (*Vicia sativa* L.) is one of the most widely distributed annual leguminous crops throughout the Mediterranean basin, western Asia and in countries of the former Soviet Union (Martiniello and Ciola, 1995; Dhima et al., 2007; Yolcu et al., 2010). It can be used for pasture or as grain legume, showing high palatability at all growth stages. Because of its high feed value for animals it is often used as grain for livestock feed (mainly lambs) and also for production of silage and hay or as green manure (Acikgoz, 1988). Common vetch has interesting traits that are desirable in organic or low input culture systems. It forms a strong fibrous root system that develops nodules at an early stage. Thus, it fixes the atmospheric nitrogen into the soil and benefits the subsequent crops (usually cereals) in both yield and quality (Papastylianou, 1999; Rinnofner et al., 2008). In addition, it is broadly used in crop rotation systems to manage diseases, weeds, improve soil fertility and contribute to increased yield and protein content in the following crops (Teasdale, 1996; Vasilakoglou et al., 2008). Furthermore, it is used in intercropping systems with cereals (Lithourgidis et al., 2007). The need to reduce external inputs in agricultural systems is a challenge for both plant breeders and farmers. In organic and low-input agriculture the concept is to utilize or develop genetic material with high adaptability to a wide range of different environments (Desclaux et al., 2008; Vlachostergios and Roupakias, 2008). However, low-input farming systems are characterized by high heterogeneity and thus a lot of the varieties developed under high-input conditions failed to satisfy farmers' demands (Dawson et al., 2008; Spiertz, 2010; Vlachostergios et al., 2011a). Therefore, many researchers claim that there is a need for varieties bred under low-input conditions (Ceccarelli, 1987; Lammerts, 2002; Murphy et al., 2007). This approach, however, has to cope with environmental heterogeneity that often complicates the identification of superior genotypes or the application of consistent selection pressure under low-input conditions (Haugerud and Collinson, 1990). An alternative way to overcome this problem is to utilize genetic diversity exploiting the potential of the crop for self regulation (Suneson, 1960; Mundt, 2002). In particular, for selffertilized crops like common vetch, variety mixtures could be applied to insert genetic diversity (Finckh et al., 1999). Bowden et al., (2001) noticed that the use of varietal mixtures can provide three main advantages: stabilization of yield (particularly when the genotype by environment interactions account for a significant variation in yield), compensation effects (when a vigorous variety compensates for a weak or injured variety) and disease control. Smithson and Lenne (1996) summarized the results from many experiments on varietal mixtures in different crops and concluded that this is a viable strategy for sustainable agriculture having the potential for improvement in productivity without sacrificing the genetic diversity. The criterion for selecting certain varieties to form varietal mixtures is an issue for discussion. Usually farmers match varieties for a restricted number of traits according to their experience in monoculture. However, the yielding ability of a variety in mixture and in pure stand is not always positively correlated because of the unpredictable interactions among genotypes in the mixtures (Ceccarelli et al., 1991; Smithson and Lenne, 1996). A general concept could be that varieties that will be evaluated as mixture components should be of high adaptability; although field experimentation is needed to suggest the best combinations (Ceccarelli, 1987; Lammerts, 2002). Cultivar mixtures, mainly in wheat and barley, have been used at varying extent in Europe and the USA (Finckh et al., 1999; Wolfe, 2001; Cowger and Weisz, 2008). Although legumes are recognized as a pivotal factor in low-input agriculture cultivar mixtures with legumes have not been studied adequately. The last decades, many farmers followed various Environmental Programs for nitrogen elimination and included vetch cultivation into their rotation schemes. However, the varieties usually cultivated were commercial pure lines which have been developed under high-input conditions, and their adaptability in low-input conditions remains unknown. Given that cultivar mixtures could serve as an alternative proposal, Vlachostergios et al. (2011b) studied the mixing ability of conventionally improved common vetch cultivars for dry matter and crude protein production and identified certain mixtures that significantly out-yielded pure stands when cultivated under low-input farming and could be recommended for low-input cultivation. It would be interesting then to investigate the mixing ability of conventionally bred common vetch cultivars for grain yield under low-input cultivation.

The objective of this work was to evaluate the performance of six common vetch cultivar mixtures composed of conventionally bred cultivars over their individual components for grain yield when grown under low-input conditions.

Results

Significant differences among entries were detected (P<0.05). Yields and entry ranks for each environment are presented in Table 3. In particular, mean yield ranged from 1.23 t ha^{-1} to 1.48 t ha^{-1} in Envt 1, from 1.19 t ha^{-1} to 1.82 t ha^{-1} in Envt 2, from 1.12 t ha^{-1} to 1.63 t ha^{-1} in Envt 3, and from 1.05 t ha^{-1} to 1.86 t ha^{-1} in Envt 4. Envt2 and Envt4 were the more productive environments as they indicated the highest mean yields. Under all environments the mean yield value of mixtures was higher, but not significant, than the mean yield value of the cultivar components. Entries toward the right of the almost vertical axis in GGE biplot analysis diagram (Fig. 1) yielded above average of the experiment. Mixtures Mix2, Mix3, Mix5 and Mix6 represented the highest-yielding mixtures, while Clt2 and Clt5 represented the highest-yielding cultivars. The yield stability of each entry was negatively associated with the length of the projection of each entry from the average environment axis (Yan, 2001; 2002). Mean yield (t ha^{-1}) of each entry across four environments, GGE bi-plot instability values and distance from the ideal entry are presented in Table 4. Among the six highest-yielding entries, Mix6 averaged across environments was more stable, followed by Clt2, Clt5, Mix5, Mix2 and Mix3 (Fig.1; Table 4). On the other hand, among the five lowest-yielding entries (located in the left of the vertical axis) Clt1 and Clt3 were more stable, followed by Mix4, Clt4 and Mix1 (Fig. 1; Table 4). In addition, cultivar Clt2 ranked closest to the ideal entry followed by Mix2, Mix5, Mix3, Mix6 and Clt5, indicating the highest stability and yield performance (Fig.1; Table 4). Furthermore, GGE biplot analysis indicated that cultivar Clt2 performed better in Envt1, Envt3 and Envt4, while Mix3 in Envt2 (Fig. 2). ME values were either positive or negative. ME ranged from -0.06 t ha^{-1} to 0.24 t ha^{-1} in Envt 1, from -0.27 t ha^{-1} to 0.32 t ha^{-1} in Envt2, from -0.14 t ha^{-1}to 0.10 t ha^{-1} in Envt3 and from -0.10 t ha^{-1} to 0.34 t ha^{-1} in Envt4. Mix 3 had significantly positive ME under Envt 1 and Envt2, Mix6 under Envt2 and Envt4 while Mix1, Mix5 had significantly positive ME under Envt4 and Envt3 respectively (Table 5). Symptoms from chocolate spot (*Botrytis* sp.) were detected only under Envt 4. Based on the rating scale 1-9 cultivars Clt1, Clt2, and Clt4 were scored as 3; Clt5 as 5; and Clt3 as 7; while Mix1, Mix2, Mix4, Mix5, and Mix6 were scored as 3, and Mix3 was scored as 5.

Discussion

Most mixtures over-yielded the average of the experiment. Among the highest-yielding mixtures, Mix2 ranked in the top under Envt1, Envt3 and Envt4 and close to the ideal entry, while Mix6, which was consisted of all cultivars, indicated the maximum – near absolute – stability among all entries. Furthermore, these mixtures had positive ME in three out of the four environments. On the other hand, Mix3 and Mix5 indicated high yield performance only under two environments, whereas mixtures Mix1 and Mix4 yielded below the average of the experiment and would be unlikely to be recommended for low-input cultivation. These observations provide evidence for functional genetic diversity in Mix2 and Mix6 that makes them suitable for low-input cultivation. However, none of the highest-yielding mixtures over-yielded cultivar Clt2 that indicated high and stable grain yield. Smithson and Lenne (1996) and Finck et al., (1999) reported that usually cultivar mixtures stabilise or even increase yield, but rarely over-yield the best cultivar component. It should be underlined however, that the pressure from diseases was mild and might have affected the final results (Mundt et al., 1995; Cowger and Weisz, 2008).

One of the main reasons that mixtures are recommended for cultivation under low-input environments is to buffer yield against increased genotype by environment interactions (Wolfe, 1985, 2000; Bowden et al., 2001; Mundt, 2002). However, our results indicated that mixtures (averaged across environments) had higher instability values than cultivars, which means greater contribution to GEI and lower stability. An explanation for this could be that although mixtures Mix2, Mix3, Mix4 and Mix5 consisted of wide adapted cultivars, those cultivars diverged for maturity and resistance to chocolate spot. Given that in the environments tested the disease pressure was nonexistent (Envt1-3) or moderate (Envt4), earliness of each cultivar component must have played a major role for mixture's performance. Moreover,

Table 1. Cultivar, experimental code, agronomical traits of cultivar components after 3-year experiments under high-input conditions and mixture's composition.

Cultivar	Code	GY ($t\ ha^{-1}$)	Height (cm)	Earliness				Resistance to *Botrytis* sp	Mixtures					
				DF	AD	GF	DM		Mix1	Mix2	Mix3	Mix4	Mix5	Mix6
BI-65	Clt1	2.34	112	131	22	29	182	Medium	X			X		X
BI-233	Clt2	2.87	103	125	21	26	172	Medium		X		X	X	X
BI-89	Clt3	2.46	110	132	19	28	179	Susceptible			X		X	X
BI-130	Clt4	2.35	108	131	23	28	182	Medium	X	X		X		X
M-6900	Clt5	2.25	111	126	22	27	175	Medium			X		X	X

DF: days to flowering; AD: anthesis duration; GF: grain filling period; DM: days to maturity

Table 2. Precipitation recorded during the growing period and soil characteristics in the four environments of the experimentation.

Environment	Precipitation (mm)	Soil characteristics						
		Sand (%)	Silt (%)	Clay (%)	OM†(mg/kg)	NO_3^- (mg/kg)	Olsen P*(mg/kg)	K(mg/kg)
Envt 1	101.1	26	29	45	13	39	12	1.5
Envt 2	178.1	48	30	22	14	26	12	0.4
Envt 3	275.2	44	34	22	18	13	26	0.6
Envt 4	311.9	34	20	46	12	32	14	1.4

†: OM: organic matter. * Olsen P.

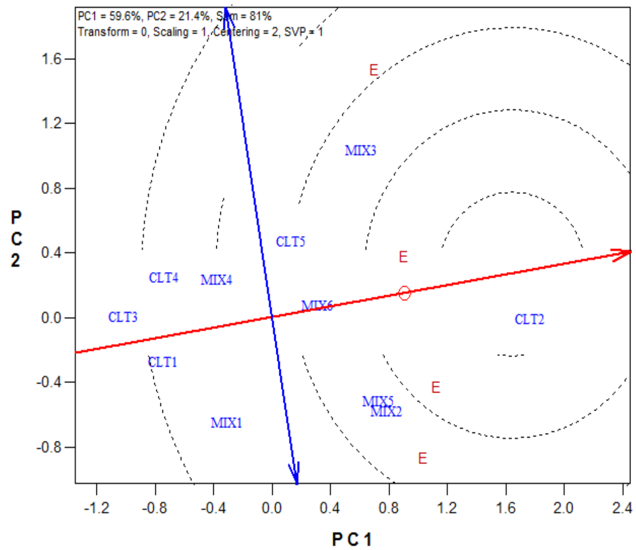

Fig 1. Distance from the ideal GGE biplot analysis for ranking 11 entries based on grain yield and stability. Entries with vertical projections further right in the one arrow horizontal axis are the highest yielding and the ones with furthest vertical projections up or down on the two arrow (vertical to the horizontal) stability line are considered less stable.

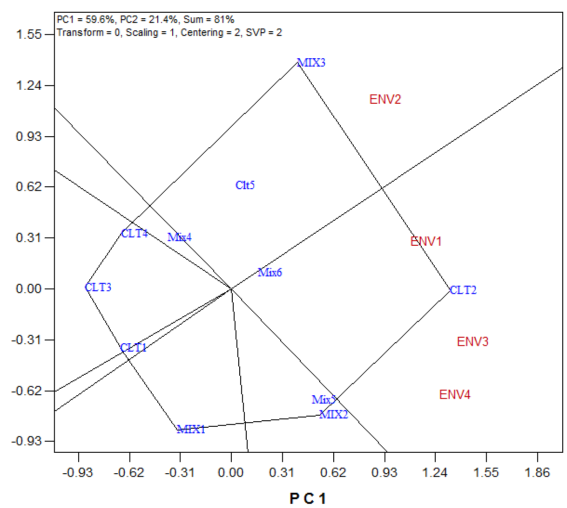

Fig 2. Polygon view of GGE biplot analysis for identification of "winner" entries based on grain yield performance of 11 entries evaluated under four environments. Environments are grouped by the perpendicular lines form the origin of the biplot to the two lines of the polygon angle as it is determined by the winner entry (CLT2).

Siddique et al., (1999, 2001) and Loss and Siddique (1994) have reported that the highest grain yield in rainfed Mediterranean areas was observed in common vetch varieties that flowered early and grain filling occurred before the dry period. In our experiment, the former observations were confirmed; the highest yielding and most stable entry was the early flowering cultivar Clt2. Nevertheless, none of the mixtures with Clt2 as a cultivar component (i.e. Mix2, Mix4, Mix5) over-yielded Clt2 pure stand. This was mainly attributed to the non-temporarily grain maturing between the cultivar components. Actually, it was observed that plants from early maturing cultivar components, like Clt2 or even Clt5, had already matured (beginning of pod dehiscence) when the other cultivar components haven't reach maturity. Thus, the contribution of early maturing cultivars in the grain yield of the mixture was diminished and therefore the yield of the mixture was reduced. This suggests that temporal maturing is a significant characteristic when blending common vetch cultivars for grain yield and should be considered with particular caution. However, it should be underlined that temporal maturing cannot be the only criterion to compose high yielding mixtures. In the present study, this was observed in Mix1, where the two cultivar components had the same earliness, but the performance of the mixture was low. Another significant point when blending common vetch mixtures is the purpose of cultivation; whether the mixture is cultivated for dry matter or for grain. Different performance was detected by the same mixtures when evaluated for dry matter production or for grain yield. A typical example was Mix4 that was one of the most stable and high yielding mixtures for dry matter (Vlachostergios et al., 2011b) and one of the lowest yielding mixtures for grain yield. Mixture effect (ME) indicates the quantitative relation between the mixture and its conventionally bred cultivar components and could be used as an index for the commercial value of the mixtures. In the

Table 3. Rank order and grain yield production (t ha^{-1}) of 11 entries and means of cultivar components and mixtures under each environment.

	Envt 1		Envt 2		Envt 3		Envt 4	
Ranking	Entry	(t ha^{-1})	Entry	(t ha^{-1})	Entry	(t ha^{-1})	Entry	(t ha^{-1})
1	Clt2	1.48	Mix3	1.82	Clt2	1.63	Clt2	1.86
2	Mix3	1.48	Clt5	1.71	Mix5	1.48	Mix5	1.80
3	Mix2	1.47	Clt2	1.67	Mix2	1.46	Mix2	1.64
4	Mix4	1.31	Mix6	1.59	Clt5	1.38	Mix6	1.61
5	Clt4	1.30	Mix4	1.45	Mix6	1.32	Mix1	1.39
6	Clt1	1.30	Mix5	1.44	Mix3	1.22	Clt5	1.36
7	Mix5	1.30	Clt4	1.35	Mix1	1.20	Mix3	1.35
8	Mix6	1.29	Clt3	1.29	Clt4	1.18	Mix4	1.29
9	Mix1	1.25	Mix2	1.23	Mix4	1.17	Clt1	1.28
10	Clt5	1.24	Mix1	1.23	Clt1	1.13	Clt3	1.17
11	Clt3	1.23	Clt1	1.19	Clt3	1.12	Clt4	1.05
Mean		1.33		1.45		1.30		1.43
$LSD_{0.05}$		0.29		0.28		0.08		0.30
CV (%)		15		13		11		14
Means								
Cultivars		1.31		1.44		1.29		1.33
Mixtures		1.35		1.46		1.31		1.51

Mix1: Clt1 & Clt4; Mix2: Clt2 & Clt4; Mix3: Clt3 & Clt5; Mix4: Clt1, Clt2 & Clt4; Mix5: Clt2, Clt3 & Clt5; Mix6: Clt1, Clt2, Clt3, Clt4 & Clt5.

present study the ME values recorded didn't indicate a stable grain yield advantage of the mixtures over their cultivar components. Therefore, before recommending a common vetch cultivar mixture for grain yield production, the benefits achieved by the mixture cultivation need to be carefully weighed against the agronomic practices of the low-input culture system. Even for high-yielding mixtures there are some practical disadvantages (i.e. added time, cost involved in mixing, etc.) that should be taken into account.

Materials and methods

Cultivar component selection

The criterion for composing the mixtures was to blend cultivars with wide adaptability under conventional environment. For this purpose, ten cultivars developed from conventional breeding programs were evaluated for significant agronomical traits under high-input conditions across 3-years (Vlachostergios et al., 2011b). The five high-yielders were selected and composed the mixtures in six different combinations (Table 1). Each year untreated seeds of the component cultivars participated in equal seed proportion to form the mixtures.

Experimental design and crop management

Field experiments were established at three locations. In particular, at the central farm of Fodder Crops and Pastures Institute (FCPI) in Larissa, (latitude 39°36′N, longitude 22°25′E) during two consecutive growing seasons (2007-08 and 2008-09), at the farm of Aristotle University (AUTH), (latitude 40°32′N, longitude 22°59′E) in 2007-08 and at the farm of the Agricultural University of Thessaly (UTH) (latitude 39°23′N, longitude 22°45′E) in 2007-08. In all sites a two-year rotation was applied consisting of durum wheat/legume (lentil, common vetch or field pea). Recommended practices appropriate to each site were followed with respect to soil preparation. No fertilizers or other agrochemicals were applied either on the previous culture (durum wheat) or on common vetch. Common vetch cultivars and their mixtures were seeded at a rate of 160 kg/ha in the last week of November in both growing seasons. The experimental design was a randomized complete block with eleven treatments (five cultivars and six mixtures) replicated four times. Individual plots consisted of six rows spaced 0.25 m apart and 4 m long and occupied 6 m^2. All plots in each replication were separated by 1 m buffer zone and replications were separated by 2 m buffer zone. Plots were kept free of weeds by implementing hand hoeing, where necessary. Satisfactory nodulation was verified by visual examination of root system. Experimental sites had different climatic conditions and soil properties (Table 2). At grain maturity, the experimental plots were hand-harvested and threshed using a stationary Wintersteiger (F. Walter and H. Wintersteiger, Ried/Innkreis, Austria) thresher in order to assess grain yield. The harvested area was 4 m^2 per plot, as only the four central rows were harvested. Visual evaluation for resistance to chocolate spot (*Botrytis* spp.) was conducted using a rating scale from 1 to 9 as follows: 1 = no lesions visible; 3 = few scattered lesions seen after careful searching; 5 = lesions common and some lesions coalesced, little defoliation; 7 = large lesions, very common and damaging, some defoliation; 9 = lesions very large, very extensive defoliation.

Data analysis

Combinations of location and culture period will be referred to as environments. Environment 1 (Envt1) represents FCPI/2007-08, Environment 2 (Envt2) represents AUTH/2007-08, Environment 3 (Envt3) represents UTH/2007-08, and Environment 4 (Envt4) represents FCPI/2008-09.

The computer program MSTAT version 1.2 (1988) was used to conduct the analysis of variance (ANOVA) for each environment and combined analysis over environments. Differences between means were compared at the 0.05 level of significance. Ranks were assigned to genotypes for grain yield.

Table 4. Mean yield (t ha^{-1}) of each entry across four environments. GGE bi-plot instability values, distance from the ideal entry and means of cultivar components and mixtures.

Entry	Mean Yield†	GGEbiplot instability value§	Distance from the ideal entry*
Clt1	1.22	0.137	2.5
Clt2	1.66	0.285	0.2
Clt3	1.19	0.181	2.8
Clt4	1.22	0.382	2.5
Clt5	1.42	0.456	1.6
Mix1	1.27	0.578	2.2
Mix2	1.45	0.685	1.2
Mix3	1.47	0.935	1.4
Mix4	1.31	0.306	2.1
Mix5	1.50	0.610	1.3
Mix6	1.45	0.034	1.4
Mean			
Cultivars	1.34	0.29	1.9
Mixtures	1.41	0.52	1.6

†: LSD *0.05*: 0.25, §: A greater value means greater contribution to GE and less stable. *A smaller value means highest mean performance and stability.

Table 5. Mixture effect (ME) of six common vetch mixtures for grain yield under each environment (Envt).

Mixtures	ME (t ha^{-1})			
	Envt 1	Envt 2	Envt 3	Envt 4
Mix1	-0.06	-0.04	0.04	0.23*
Mix2	0.08	-0.27	0.05	0.19
Mix3	0.24*	0.32*	-0.03	0.11
Mix4	-0.05	0.05	-0.14*	-0.10
Mix5	-0.02	-0.11	0.10*	0.34
Mix6	-0.02	0.14*	0.03	0.27*

*Significant at the 0.05 probability level, $ME = Y_m - Y_c$ (Y_m: mixture's yield; Y_c : average yield of mixture's components).

Mixture effect (ME) was calculated as the difference between the yield of each mixture and the average yield of its components:

$$ME = Y_m - Y_c ,$$

where Y_m is the yield of the mixture and Y_c is the average yield of its components. Mixture Effect was tested for its significance with t-test ($P< 0.05$)

To determine stability across environments, a genotype and genotype x environment (GGE) biplot analysis was conducted using GGE Biplot Pattern Explorer software (Yan, 2001; 2002). The GGE biplot model provides breeders with a complete visual evaluation of all aspects of the GxE interaction by creating a biplot that simultaneously represents both mean performance and stability. This model decomposes G plus GxE effects through singular value decomposition (SVD) into two or more principal components. Thereby, it removes the noise caused by the environment main effect (E) and emphasizes the two components of genotype effects (G) and GxE, which are more meaningful to breeders. The GGE biplot measures the distance of each genotype from the "ideal genotype", which is defined as the virtual genotype that has the highest mean performance and stability (Yan, 2001; Yan and Kang, 2003).

Conclusion

The present work indicated that one could isolate common vetch cultivars bred under high-input conditions with considerable mixing ability to compose mixtures for grain production under low input cultivation. However, earliness and temporal maturing of common vetch cultivar components, as well as practical difficulties should be carefully evaluated when composing common vetch cultivar mixtures for grain yield.

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