



Characterization of grass pea (*Lathyrus sativus* L.) germplasm collections for beneficial agronomic traits

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Abstract

At ICARDA, germplasm collections obtained from five countries namely, Bangladesh, Ethiopia, Syria, Nepal and Pakistan were grouped in to seven sets of trials (BANG1, BANG2, ICARDA, ETH1, ETH2, NEP, PAK) and evaluated in two experimental locations, Tel Hadya and Breda over eight years. Characterization was done for the most desirable agronomic traits such as days to first flowering, days to 95% maturity, biological yield, seed yield, hundred seed weight and harvest index. Significant genotypic and interaction effect were found for the expression of the traits. Substantial amount of variability was revealed in the germplasm collections for most of the traits. High broad sense heritability was observed for days to first flowering and hundred seed weight indicating early phenotypic selection of these two traits. The stability over years for the seed yield (in terms of the CV), identified promising accessions such as ILG2178 in BANG1 and ILG1721, ILG1632, ILG1624, ILG1540 and ILG1501 for future breeding.

Key words: Breeding, characterization, germplasm, *Lathyrus sativus*, Legume, Variation, BLUP

Introduction

Grass pea (*Lathyrus sativus* L.), commonly known as chickling vetch is a cool season legume crop (2n=14) (Talukdar 2009; McCutchan 2003). It is traditionally valued as a food and fodder legume. It offers rich source of seed protein (18-34%) for the human diet; and thus, it plays an important role in food protein supply of the poor people living in many developing nations (Nazrul and Shaheb 2015; ICARDA 2015; Dixit et al. 2016). Also, it has a great scope for cultivation in the drought prone areas; there has been an increasing interest in recent times for grass pea cultivation to counter the effect of global climate

change. Eventually, the area of grass pea cultivation is expected to increase in the near future (Dejene and Lijalem, 2012; Almeida et al. 2015).

However, presence of neurotoxin, β -N-oxalyl-L-, α -diaminopropionic acid (ODAP) in grass pea seeds causes paralysis of leg muscles in human beings (Neurolathyrism), when it is consumed continuously as a major portion for a prolonged period of time (McCutchan 2003; Kumar et al. 2011; Kumar et al. 2013). Besides, there are some undesirable agronomic crop characteristics which limits the widespread cultivation of grass pea in various environmental conditions (Rybiński 2003). Numerous studies had been done to address the problem of seed ODAP content in the past three decades and it was extensively reviewed by Kumar et al. (2011), Kumar et al. (2013) and Dixit et al. (2016). But, very few breeding efforts were done for the improvement of agronomic traits (Ahmadi et al. 2015; Rizvi et al. 2016; Abate et al. 2018). In order to develop varieties with desirable agronomic traits determination of genetic variation in the existing germplasm is necessary.

The International Center for Agricultural Research in the Dry Areas (ICARDA) holds large germplasm collection of *Lathyrus* spp. Using this precious resource, ICARDA collaborated with national breeding programmes and developed grass pea varieties with low ODAP content. But, it has not been completely characterized for agronomic traits. Availability of such information would be useful to identify new sources for grass pea improvement. With this background, the present work was carried out to (1) evaluate grass

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pea germplasm collections of ICARDA at two different locations over multiple years (2) assess the genetic variability and heritability of the agronomic traits (3) identify the most promising sources with desirable agronomic traits for future breeding.

Materials and methods

An extensive multi-environmental trial was conducted with 702 grass pea germplasm accessions at two research stations namely Breda and Tel Hadya, located in northern Syria for eight years (1999-2006). Based on the origin, the entire germplasm was grouped in to seven different sets of trials namely, BANG1 and BANG2 from Bangladesh, ETH1 and ETH2 from Ethiopia, ICARDA from Syria, NEP from Nepal and PAK from Pakistan. The total number of accessions across the trials varied from 49 to 225. ILG347 was used as a local check in all trials except in ETH2 and ICARDA; ILG587 was used as a local check in ETH2 and ILG431 was used as a local check in ICARDA. All trials were conducted in a simple lattice design. Except for BANG2 and ETH2, all trials were conducted at two locations; and these two were conducted only in Tel Hadya because of low seed availability. During the crop growing period, data collection was done for the agronomic traits including days to first flowering (DF), days to 95 % maturity (DM), biological yield (BY), seed yield (SY) and hundred seed weight (HSW). Harvest index (HI) was estimated as a ratio of SY to the BY (AghaAlikhani et al. 2014).

A combined analysis was carried out for the data collected over different environments. Variance components of genotype (G), genotype x location (GxL) and genotype x year within locations (GxYxL) and pooled error variance were estimated by Residual Maximum Likelihood (REML) approach. It considered location as a “fixed” effect and replications and blocks within replications within location – year combinations as “random” effects. From the analysis, the Best Linear Unbiased Predictors (BLUP) values were attained and utilized to derive the trial means and ranges for all traits. The broad-sense heritability (h^2_b) of each trait was calculated by using the formula as follows:

$$h^2_b = \sigma^2_g / (\sigma^2_g + \sigma^2_{gl}/L + \sigma^2_{gy(l)}/(yl) + \sigma^2_e/(ylr))$$

where, σ^2_g = genetic variance, σ^2_{gl} = genotype by location interaction variance component, $\sigma^2_{gy(l)}$ = genotype x year interaction within location variance component, σ^2_e = pooled error variance, l = number of locations, y = number of years within a given location and r = number of replications. According to Robinson

et al. (1949) the heritability estimates were categorized as low (<0.30), moderate (0.31-0.60) and high (>0.60). As a measure of stability over the years for seed yield, coefficient of variation (CV) was obtained in terms of BLUPs of genotype x year interaction within locations for each genotype using the quadratic mean of such CVs over the locations (Imtiaz et al. 2013).

Results and discussion

The combined analysis revealing differences in the significance level (P-values) of genotypic effect (G), interaction effect across locations (GxL) and year within locations (GxYwL) is presented in Table 1. Significant genotypic differences were found for all traits across the trials, except DM in BANG1 and PAK and SY in ETH2. The genetic variability was set at the boundary at zero to avoid an invalid estimate as negative values and the interaction effects contributed as the major source of variation for days to maturity and harvest index in ETH1. The GxL interaction was found significant for DF in ICARDA and PAK; DM in BANG1, ETH1, ICARDA, NEP and PAK; HSW in BANG1, BY in PAK and HI in ETH1. Furthermore, the GxYwL interaction was found significant for all traits across the trials except for DM in ETH1; SY and HI in ICARDA and BY in PAK. A study conducted by Polignano et al. (2009) also demonstrated genotype x environment effects on these traits.

The minimum and maximum values of the traits presented in Table 2, identified BANG2 (79.7 days) for early flowering, ETH2 for early maturity (139.3 days) and large seeds (17.52g 100 seeds⁻¹); BANG2 for high SY (1721 Kg/ha) and high BY (5783 Kg/ha) and ETH1 and NEP for HI (0.42) as beneficial sources (Table 2). The variation found for earliness and BY in BANG2 would facilitate breeders to develop varieties which could benefit short season Mediterranean environments (Hanbury et al. 1995) and improve grass pea for fodder purpose. High broad sense heritability of DF and HSW across all trials, indicate the influence of additive gene action and early phenotypic selection for these two traits (Table 1). However, other traits have obtained similar changes in heritability values as shown by Ahmadi et al. (2015) during the evaluation over different environmental conditions.

The five most promising accessions for each individual trait were selected based on BLUP (Best Linear Unbiased Predictor) values and presented in Table 3. Particularly, accessions such as 2537, 2504, 2517, 2541 and 2577 in BANG2 had early flowering

Table 1. Estimates of components of variance, their standard errors and broad sense heritabilities of the traits evaluated across the trials

Trials	Traits	σ^2_g		σ^2_{gl}		$\sigma^2_{gy(l)}$		σ^2_e		h^2_b	
		Estimate	S.E.	Estimate	S.E.	Estimate	S.E.	Estimate	S.E.	Estimate	S.E.
BANG 1	DF	1.205***	0.237	0.044	0.115	1.492***	0.174	3.152	0.154	0.7857	0.05037
	DM	0.29	0.258	0.909***	0.296	1.151***	0.306	7.902	0.364	0.2399	0.1836
	HSW	0.0678***	0.0177	0.0259*	0.0136	0.0349**	0.0154	0.369	0.0184	0.6605	0.08679
	SY	8450***	1879	0	Bound ^a	15945***	2659	59849	2820	0.6696	0.05149
	BY	99167***	17565	0	Bound ^a	83936***	12978	284922	13439	0.8281	0.02652
BANG 2	HI	0.000079***	0.000024	0	Bound ^a	0.000153**	0.000052	0.00139	0.000065	0.5055	0.07759
	DF	2.621***	0.294	NA ^b		NA ^b		3.314	0.135	0.8558	0.01518
	DM	4.179***	0.469	NA ^b		NA ^b		6.646	0.251	0.8558	0.01498
	HSW	0.6892***	0.0742	NA ^b		NA ^b		0.566	0.0281	0.8906	0.01218
	SY	15210***	1967	NA ^b		NA ^b		51391	1949	0.751	0.02608
ETH 1	BY	185828***	20679	NA ^b		NA ^b		260971	9921	0.8638	0.01419
	HI	0.000102***	0.000026	NA ^b		NA ^b		0.00162	0.000061	0.4011	0.06363
	DF	3.595***	0.574	0	Bound ^a	3.072***	0.235	4.178	0.177	0.9006	0.01491
	DM	0	Bound ^a	3.72***	0.447	1.799	2.42	6.328	0.251	9.29E-08	1.01E-08
	HSW	0.168***	0.0273	0.0058	0.0065	0.0368**	0.0138	0.424	0.0171	0.8905	0.02267
ETH 2	SY	2005**	832	1015	878	8171***	1832	53343	2122	0.4146	0.1236
	BY	26508***	6275	0	Bound ^a	24677**	10515	264355	12923	0.6283	0.05804
	HI	0	Bound ^a	0.000551***	0.000151	0	Bound ^a	0.00516	0.000188	0.0000019662.913E-07	
	DF	4.165***	0.93	NA ^b		NA ^b		6.89	0.534	0.8145	0.03699
	DM	12.329***	2.373	NA ^b		NA ^b		6.481	0.447	0.9423	0.01122
ICARDA	HSW	2.407***	0.4468	NA ^b		NA ^b		0.584	0.04	0.9755	0.0048
	SY	1895	1195	NA ^b		NA ^b		46644	3255	0.3108	0.14
	BY	79846***	18725	NA ^b		NA ^b		213226	14930	0.7846	0.04248
	HI	0.00038***	0.000091	NA ^b		NA ^b		0.00146	0.000102	0.7767	0.0444
	DF	2.768***	0.485	0.269*	0.161	2.296***	0.275	6.608	0.269	0.8381	0.03343
NEP	DM	1.303***	0.312	0.943***	0.212	0.579**	0.207	6.846	0.261	0.6437	0.07342
	HSW	0.8457***	0.1264	0	Bound ^a	0.099***	0.0215	0.643	0.0256	0.9679	0.004822
	SY	1786**	746	0	Bound ^a	3534	2673	92280	3544	0.3652	0.09886
	BY	8008*	4580	0	Bound ^a	28478*	14364	362582	17865	0.2763	0.1173
	HI	0.000082*	0.000047	0	Bound ^a	0.000026	0.000154	0.00443	0.000211	0.2673	0.115
PAK	DF	3.795***	0.893	0.054	0.199	2.997***	0.383	5.032	0.321	0.8938	0.03193
	DM	3.09***	0.9	1.359***	0.452	2.384***	0.394	7.124	0.409	0.7418	0.07667
	HSW	1.1117***	0.2429	0	Bound ^a	0.3241***	0.0488	0.781	0.047	0.9561	0.009551
	SY	9056***	2619	1451	1315	16815***	2274	34166	2019	0.7521	0.07511
	BY	222590***	50995	0	Bound ^a	118949***	17720	228654	15864	0.913	0.01914
PAK	HI	0.000194*	0.000089	0.000108	0.000094	0.00068***	0.000121	0.0016	0.00011	0.5069	0.149
	DF	2.167***	0.532	0.657**	0.249	2.759***	0.265	3.511	0.191	0.7623	0.06163
	DM	0.007	0.376	2.042***	0.528	2.658***	0.373	7.261	0.372	0.004638	0.2592
	HSW	0.1133***	0.0273	0.0169	0.0123	0.0414*	0.0233	0.568	0.0294	0.7814	0.05803
	SY	5738***	1417	0	Bound ^a	13160***	1736	32746	1694	0.7445	0.04862
PAK	BY	65188*	29130	79687**	29485	46659	28906	622453	36342	0.4739	0.1415
	HI	0.000217***	0.000064	0.000007	0.000054	0.00061***	0.000087	0.00127	0.000076	0.6509	0.09286

*, ** and *** significant at 5%, 1% and 0.1% respectively; a-Variance components estimates kept at the boundary when restricted to positive range. b-Data not available

Table 2. Summary of trial means, standard deviation (SD), co-efficient of variation (CV) and the range derived from the BLUP values through REML analysis

Trial code	Traits	Mean	SD	CV (%)	Minimum	Maximum
BANG1	DF	88.60	1.10	1.24	86.30	91.20
	DM	141.80	0.70	0.49	140.00	143.30
	HSW(g)	7.77	0.25	3.22	7.21	8.528
	SY (Kg/ha)	1496.00	83.10	5.55	1294.00	1655.00
	BY (Kg/ha)	3866.00	300.10	7.76	3157.00	4570.00
	HI	0.38	0.007	1.84	0.36	0.41
BANG2	DF	83.60	1.60	1.91	79.70	92.30
	DM	144.20	2.00	1.39	140.30	154.40
	HSW(g)	7.76	0.79	10.18	6.50	13.78
	SY (Kg/ha)	1099.00	115.80	10.54	880.00	1721.00
	BY (Kg/ha)	3176.00	422.30	13.30	2413.00	5783.00
	HI	0.35	0.01	2.86	0.32	0.37
ETH1	DF	102.40	1.90	1.86	95.00	106.60
	DM	154.10	1.20	0.78	149.80	156.20
	HSW(g)	8.95	0.40	4.47	7.99	10.40
	SY (Kg/ha)	1184.00	42.80	3.61	1075.00	1294.00
	BY (Kg/ha)	3587.00	134.60	3.75	3312.00	4087.00
	HI	0.32	0.01	3.13	0.31	0.42
ETH2	DF	93.10	2.00	2.15	86.70	97.10
	DM	151.10	3.50	2.32	139.30	159.20
	HSW(g)	9.32	1.54	16.52	7.28	17.52
	SY (Kg/ha)	1256.00	39.90	3.18	1182.00	1367.00
	BY (Kg/ha)	3852.00	275.00	7.14	3418.00	4814.00
	HI	0.32	0.02	6.25	0.26	0.36
ICARDA	DF	99.80	1.70	1.70	95.35	103.08
	DM	151.60	1.30	0.86	147.70	153.90
	HSW(g)	11.28	0.91	8.07	9.43	15.34
	SY (Kg/ha)	1224.00	28.1.00	2.30	1162.00	1410.00
	BY (Kg/ha)	4831.00	55.8.00	1.16	4687.00	4964.00
	HI	0.25	0.005	2.00	0.24	0.29
NEP	DF	97.60	1.90	1.95	95.10	104.40
	DM	148.80	1.90	1.28	146.10	156.80
	HSW(g)	7.70	1.05	13.64	6.86	12.49
	SY (Kg/ha)	982.00	98.30	10.01	851.00	1358.00
	BY (Kg/ha)	2834.00	465.00	16.41	2380.00	4857.00
	HI	0.38	0.015	3.95	0.33	0.42
PAK	DF	96.80	1.60	1.65	93.50	103.70
	DM	149.60	1.00	0.67	147.80	153.50
	HSW(g)	7.97	0.33	4.14	7.36	8.87
	SY (Kg/ha)	1025.00	74.20	7.24	863.00	1174.00
	BY (Kg/ha)	3528.00	285.00	8.08	2875.00	4313
	HI	0.32	0.014	4.38	0.27	0.34

Table 3. The list of desirable accessions selected based on BLUP values (the lowest five in ascending order for DF and DM and the highest five in ascending order for HSW, SY, BY and HI).

Trials	DF		DM		HSW (g)		SY (kg/ha)		CV% (rank) ^s	BY (Kg/ha)		HI	
	ILG no.	Est	ILG no.	Est	ILG no.	Est	ILG no.	Est		ILG no.	Est	ILG no.	Est
BANG1	2263	86.89	1873	140.7	2178	8.239	2301	1655	29.5(21)	2112	4348	1873	0.4055
	2206	86.85	1823	140.5	2092	8.183	2123	1644	30.1(27)	2290	4342	2188	0.398
	2122	86.72	2096	140.5	2123	8.181	2074	1617	29.1(15)	2167	4290	1844	0.3976
	2196	86.62	1872	140.4	2175	8.167	2178	1616	27.9(2)	2284	4289	2301	0.3967
	2088	86.32	1844	140	2265	8.122	2112	1615	30.5(38)	2178	4287	2088	0.3957
	LC	88.33		142.7			8.528				4570		
	SE±	7.105		6.331		0.58		182		462			0.0219
BANG2	2577	80.5	2541	141.1	2816	13.775	2997	1721	31.1(3)	2997	5783	2375	0.3703
	2541	80.44	2426	141	2404	13.578	3021	1632	29.3(2)	2999	5399	2854	0.3696
	2517	80.31	2405	140.5	2405	12.271	2999	1624	29.1(1)	3021	5360	2541	0.3695
	2504	79.76	2404	140.4	2426	10.954	3026	1540	32.1(5)	3026	4948	2333	0.3643
	2537	79.73	2758	140.3	2431	9.288	3027	1501	32.0(4)	3027	4393	2433	0.3643
	LC	83.88		143.9			7.556		1132		3362		
	SE±	9.221		8.135		0.55		189.2		552.1			0.01804
ETH1	402	98.7	400	150.9	426	10.395	440	1294	52.2(5)	440	4087	438	0.4158
	385	98.6	483	150.3	483	10.013	486	1289	52.6(8)	413	3917	385	0.3287
	455	98.4	422	150.1	468	9.825	413	1282	52.1(3)	486	3883	386	0.3285
	393	98.2	387	150	455	9.771	421	1273	51.3(1)	422	3836	477	0.3272
	400	97.1	468	149.8	387	9.738	410	1266	54.5(28)	421	3826	414	0.3264
	LC	95		150.2			9.333		1207		3726		
	SE±	4.954		3.943		0.2878		180.5		725.8			0.02697
ETH2	ETH-42	90.42	ETH-35	147.8	ETH-29	17.519	PA-K-208	1367	47.1(2)	736	4814	PA-K-209	0.3574
	ETH-39	90.04	ETH-34	146.7	ETH-42	14.222	ETH-20	1329	51.5(11)	WIR-70	4710	PA-K-208	0.3559
	PA-K-290	89.8	PA-K-100	140.4	ETH-34	13.562	WIR-70	1327	50.5(7)	WIR-26	4678	ETH-34	0.3506
	ETH-7	89.73	PA-K-208	139.9	PA-K-100	12.424	ETH-11	1322	53.3(31)	WIR-475	4362	ETH-46	0.3471
	ETH-19	89.62	PA-K-209	139.3	PA-K-209	10.847	PA-K-33	1310	53.0(26)	PA-K-33	4275	ETH-48	0.3449
	LC	86.71		146.2			8.824		1266		4041		
	SE±	7.663		5.348		0.3536		237.7		603.9			0.02447
ICARDA	690	97.03	708	149.4	701	15.34	712	1410	42.0(1)	676	4964	712	0.2869
	701	96.51	675	149.1	670	13.25	692	1255	47.6(3)	672	4963	689	0.2573
	675	95.74	701	149.1	691	12.89	728	1255	47.9(8)	736	4957	663	0.2572
	311	95.44	311	149	519	12.72	731	1254	47.9(7)	711	4947	733	0.257
	521	95.35	521	147.7	521	12.66	733	1253	48.0(10)	735	4931	717	0.2563
	LC	98.77		150.9			10.81		1223		4855		
	SE±	4.687		4.029		0.3007		132.7		613.4			0.01865
NEP	1976	100.12	1974	149.7	170	12.241	273	1357.9	39.0(1)	273	4857	1972	0.419
	1977	97.09	1976	148.7	1894	7.88	170	1241.1	43.7(4)	170	4426	1897	0.409
	1978	97.91	1977	148.1	1896	7.589	1964	1101	39.2(2)	1894	3194	1964	0.401
	1989	100.18	1978	149	1897	7.127	1894	1082.2	46.7(6)	1937	3088	1977	0.3989
	273	104.37	1989	148.4	1899	7.299	1948	1081.7	50.8(22)	1959	3067	1916	0.3976
	LC	95.9		151			8.414		1164.4		3885		
	SE±	5.044		4.003		0.3008		130		453.5			0.02037
PAK	1781	94.68	1760	148.4	952	8.872	1777	1152	47.3(1)	1771	4313	1798	0.342
	1775	94.67	1804	148.4	1761	8.684	1784	1151	48.0(4)	945	3993	957	0.3415
	1798	94.14	1801	148.3	1760	8.675	945	1146	47.4(2)	936	3975	1812	0.3401
	1761	94.11	1761	147.8	1809	8.592	1794	1139	51.2(20)	11	3919	1769	0.3399
	1785	93.48	1797	147.8	1797	8.513	1776	1130	50.1(14)	937	3907	1765	0.3391
	LC	94.87		150.1			8.592		1174		4032		
	SE±	4.978		3.713		0.2932		143.6		605.1			0.02854

SE= Estimated standard error. CV%= Coefficient of variation of BLUPs across years (or quadratic mean of CVs for two locations)

(<80.5 days) than the local check 347 and accessions PAK208 and PAK209 in ETH2 demonstrated early maturity (139 days) than the local check 587. Accessions namely 2816 and 2404 in BANG2, ETH-29, ETH-42, ETH-34 in ETH2 and 701 and 670 in ICARDA had more than 13g of HSW. Accessions including 2997, 3021 and 2999 in BANG2 were identified as high yielding accessions. Accessions namely 2997, 2999 and 3021 in BANG2 recorded high BY and accessions including 1873 in BANG1, 438 in ETH1, 1972, 1897 and 1964 in NEP had demonstrated high HI. Interestingly, the accession 2997 was found to have multiple beneficial traits with high SY (1721Kg/ha), BY (5783Kg/ha) and HSW (13.78g). It would be pertinent to mention that the focus of the present study was not to study the adaptation of the accessions to the repeatable bio-physical environments such as locations. Furthermore, data from too few locations, two in the present study, restricted the scope of the application of procedures such as AMMI (additive main effects and multiplicative interaction) model and GGE biplot (Gauch, 2006). However, the temporal variation or stability over years were examined for the seed yield in terms of the CV. The top five accessions for seed yield were also stable across years with relatively low CV values using rank values less than five, e.g., ILG2178 had the second lowest CV in BANG1 (Table 3). The five high yielding accessions were also most stable (lowest CV or ranks 1-5) in BANG2. In the remaining trials, one to three accessions had CV ranks within five. The diverse and promising accessions identified in the present study could be used as a donor in future breeding.

Authors' contribution

Conceptualization of research (AS); Designing of the experiments (AS, MS); Contribution of experimental materials (AS, AMA); Execution of field/lab experiments and data collection (AS, HN); Analysis of data and interpretation (AS, MS, KR); Preparation of manuscript (KR, AS, MS).

Declaration

The authors declare no conflict of interest.

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