Euphytica (2009) 165:279–291
DOI 10.1007/s10681-008-9786-2

Phenotypic diversity in cold-tolerant peanut (*Arachis hypogaea* L.) germplasm

H. D. Upadhyaya · L. J. Reddy · S. L. Dwivedi · C. L. L. Gowda · S. Singh

Received: 19 August 2007 / Accepted: 7 August 2008 / Published online: 2 September 2008

Abstract Tolerance to low temperature is an important prerequisite for optimal performance of peanut (*Arachis hypogaea* L.) in a number of temperate peanut-growing environments. One hundred fifty-eight peanut accessions belonging to five botanical types, known to be tolerant to low temperature (12°C) at germination, were evaluated for phenotypic diversity for 15 morphological traits in the 2001 rainy season and for 15 agronomic and two seed quality traits in the 2001 rainy and 2001/2002 post-rainy seasons. Analysis of data, using the residual maximum-likelihood approach indicated that variance components due to genotypes were significant for all traits in the rainy and for all but two traits in the post-rainy season. Clustering based on scores of nine principle components delineated four clusters. The cold-tolerant genotypes and the standard control cultivars in the four clusters differed in mean, variance, and range both during rainy and post-rainy seasons for a range of agronomic traits, indicating the diversity among the clusters. The cold-tolerant accessions were superior to control cultivars for several agronomic traits compared with their respective controls in both the rainy and post-rainy seasons, so their use in breeding should result in genetically diverse cold-tolerant high-yielding peanut cultivars.

Keywords Groundnut · Cold tolerance · Genetic diversity · Principal component analysis

Introduction

Peanut (*Arachis hypogaea* L.), also known as groundnut, is an important tropical legume grown for both oil production and human food, as it provides a good source of energy, protein, minerals, and vitamins. Peanut production environments are characterized by a warm, frost-free period of at least 90 days (Bunting et al. 1985), with mean temperatures between 24°C and 33°C, which is the optimum range for growth and dry-matter production (Ketring 1984). The peanut plant shows maximum growth at 28°C but experiences severe metabolic perturbations below 12°C (Bell et al. 1994a). Low temperature results in slow growth of both hypocotyl/radicle and epicotyl (Ketring et al. 1982). Night temperature determines both leaflet CO_2 exchange rate, regardless of day temperature, and the efficiency of use of intercepted photosynthetically active radiation (Sinclair et al. 1993; Bell et al. 1994b). Low soil temperature delays pod initiation, and reduces number of mature pods/seeds, and seed weight (Golombok and Johanson 1997).

H. D. Upadhyaya (✉) · L. J. Reddy · S. L. Dwivedi · C. L. L. Gowda · S. Singh
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, India
e-mail: h.upadhyaya@cgiar.org

The importance of cold tolerance in peanut is well recognized for specific production environments in North America (Benedict and Ketring 1972; Singleton and Pattee 1989; Bell et al. 1994a), Australia (Bell et al. 1991), India (Bhagat et al. 1992), and China (Fu et al. 1988). A few cold-tolerant early-maturing cultivars with ability to germinate in cooler soils have been released in Canada (Agriculture Canada 1984, 1989). The low temperatures (<18°C) at sowing in the winter peanut crop in India result in slow seedling emergence and poor plant stand. Delay in seedling emergence extends crop duration beyond 120 days, exposing the crop to high temperatures at reproductive phase and pod damage due to early onset of monsoon rains (Bhagat et al. 1988). Poor germination due to low temperatures in spring-sown peanut crop has also been reported in China, Nepal, and Vietnam (Fu et al. 1988; Koirala 1996; Dan and Hong 1996). Identification and incorporation of cold tolerance are therefore important peanut breeding objectives in these countries.

Upadhyaya et al. (2001) screened 1,704 peanut core collection accessions (Upadhyaya et al. 2003) and four control cultivars (Gangapuri, M13, ICGS 44, and ICGS 76), using rolled paper towel testing (Ellis et al. 1985) for ability to germinate in an incubator set at 12°C day-night temperature. Seeds of *hypogaea* and *hirsuta* were treated with ethrel (2-chloroethylphosphonic acid) to break seed dormancy prior to cold-tolerant test. A sufficient quantity of distilled water was added to the tray so as to keep wet the 3–4 cm of paper rolls. The number of germinating seeds was recorded at 10 (*fastigiata*, *vulgaris*, *aequatoriana*, and *peruviana* types) and 15 (*hypogaea* and *hirsuta* types) days after incubation. The *hypogaea* types that showed less than 70% germination were retested, with the seed stored for at least 6 months, to avoid discrepancies that could arise due to seed dormancy. This experiment was repeated with the same number of entries during the 2000/2001 post-rainy season. In both seasons, accessions that showed 80% or higher germination for one seed source but not less than 70% from either seed source were considered tolerant to low temperature at germination. Based on two seasons' evaluation, 158 peanut core collection accessions were identified as tolerant to low temperature (12°C), on the basis of 80% or higher germination for the best seed source and not less than 70% from the second source. Mean percentage seed germination in cold-tolerant entries ranged from 76% to 96%, compared with 36% to 55% in controls (M13, ICGS 44, and ICGS 76); the fourth control, Gangapuri, had 87% germination at 12°C (Table 1).

The present study was done to characterize phenotypic diversity for morphological and agronomic traits in the 158 cold-tolerant germplasm to identify genetically diverse accessions for use in peanut breeding to improve cold tolerance at germination.

Materials and methods

One hundred fifty-eight cold-tolerant peanut accessions, representing five botanical types (4 *aequatoriana*, 103 *fastigiata*, 11 *peruviana*, 5 *vulgaris*, and 35 *hypogaea*) and four released Indian control cultivars (Gangapuri, M13, ICGS 44, and ICGS 76) were evaluated for 15 morphological traits in field plantings in the 2001 rainy season and for 15 agronomic and two seed quality traits in the 2001/2002 post-rainy season at ICRISAT, Patancheru, India. Gangapuri (ICG 2738) belongs to subsp. *fastigiata* var. *fastigiata* (Valencia type) and matures in about 100 days. ICGS 44 (ICG 13941) belongs to subsp. *fastigiata* var. *vulgaris* (Spanish type), matures in about 120 days, and is adapted to the irrigated post-rainy season. Both M13 (ICG 156) and ICGS 76 (ICG 13942) belong to subsp. *hypogaea* var. *hypogaea* (Virginia type), mature in 120–135 days, and are adapted to rainy season conditions.

The experiment was conducted in an alpha design (Paterson and Williams 1976) with two replications in the rainy season and three replications in post-rainy season. Each accession was sown in a one row plot of 4 m length, with 60 cm between rows and 10 cm between plants in both the seasons. Morphological descriptors used included growth habit, branching pattern, stem color, stem hair, leaflet color, leaflet shape, leaflet hair, flower color, streak color on flower, peg color, seeds per pod, pod beak, pod constriction, pod reticulation, and primary seed color (IBPGR and ICRISAT 1992). Ten mature pods were randomly selected to record data on pod beak, constriction, and reticulation. Days to emergence, days to 50% flowering, pod yield per plot, pod length and width, seed length and width, and shelling

Table 1 Identity, country of origin, and germination (%) at 12°C in 1999/2000 and 2000/2001 post-rainy seasons harvested seeds in 158 cold-tolerant germplasm and control cultivars in peanut

Identity	Origin	Germination (%)		
		1999/2000 post-rainy	2000/2001 post-rainy	Mean
var. *aequatoriana*				
ICG 7898	Ecuador	86	78	82
ICG 12553	Ecuador	90	86	88
ICG 12625	Ecuador	88	78	83
ICG 12719	Ecuador	84	76	80
var. *fastigiata*				
ICG 115	India	86	100	93
ICG 282	USA	98	98	98
ICG 318	Brazil	82	88	85
ICG 376	Argentina	94	84	89
ICG 389	South Africa	92	88	90
ICG 397	USA	94	82	88
ICG 398	USA	92	80	86
ICG 445	Tanzania	82	96	89
ICG 457	USA	92	86	89
ICG 1158	India	92	94	93
ICG 1256	Uganda	86	100	93
ICG 1274	Indonesia	84	92	88
ICG 1298	Unknown	84	100	92
ICG 1384	Tanzania	86	98	92
ICG 1399	Malawi	92	70	81
ICG 1683	South Africa	94	72	83
ICG 1796	Senegal	94	98	96
ICG 1824	Zaire	90	84	87
ICG 1899	Uganda	92	98	95
ICG 1908	India	90	76	83
ICG 2039	Unknown	82	70	76
ICG 2057	China	88	94	91
ICG 2145	Sudan	90	76	83
ICG 2158	Uganda	98	75	87
ICG 2159	Sierra Leone	88	88	88
ICG 3125	Sudan	90	80	85
ICG 3219	Tanzania	88	94	91
ICG 3477	India	86	100	93
ICG 3510	Argentina	90	72	81
ICG 3726	India	92	96	94
ICG 3779	Tanzania	86	72	79
ICG 4087	USA	88	90	89
ICG 4670	Sudan	96	92	94
ICG 4788	Benin	82	92	87

Table 1 continued

Identity	Origin	Germination (%)		
		1999/2000 post-rainy	2000/2001 post-rainy	Mean
ICG 4890	Argentina	94	98	96
ICG 4992	USA	94	92	93
ICG 5094	Brazil	84	86	85
ICG 5475	Kenya	94	88	91
ICG 5609	Sri Lanka	90	92	91
ICG 5964	Zimbabwe	86	92	89
ICG 6022	Sudan	96	92	94
ICG 6148	USA	98	94	96
ICG 6203	Zimbabwe	86	90	88
ICG 6220	Brazil	88	94	91
ICG 6221	Brazil	86	84	85
ICG 6340	Honduras	90	90	90
ICG 6421	Malawi	86	88	87
ICG 6565	Unknown	84	96	90
ICG 6570	Unknown	96	100	98
ICG 6706	Brazil	90	84	87
ICG 6725	Argentina	90	94	92
ICG 6878	Argentina	98	98	98
ICG 6888	Brazil	94	100	97
ICG 7005	Brazil	88	100	94
ICG 7013	India	86	92	89
ICG 7285	Zimbabwe	98	94	96
ICG 7352	Peru	94	96	95
ICG 7355	Paraguay	90	96	93
ICG 7777	Unknown	84	98	91
ICG 7812	Brazil	82	86	84
ICG 7884	Israel	96	92	94
ICG 7905	Zimbabwe	94	88	91
ICG 7929	Paraguay	94	100	97
ICG 7978	Russia & CIS	92	100	96
ICG 8360	Thailand	84	98	91
ICG 8485	Zimbabwe	84	74	79
ICG 8514	South Africa	84	84	84
ICG 8517	Bolivia	86	94	90
ICG 8570	Argentina	92	88	90
ICG 9141	Zaire	88	96	92
ICG 9144	Syria	84	98	91
ICG 9929	Zimbabwe	88	96	92
ICG 10075	Peru	90	70	80
ICG 10092	Zimbabwe	88	76	82
ICG 10371	Nigeria	84	92	88
ICG 10402	USA	84	78	81

Table 1 continued

Identity	Origin	Germination (%)		
		1999/2000 post-rainy	2000/2001 post-rainy	Mean
ICG 10481	Venezuela	82	82	82
ICG 10495	Paraguay	90	72	81
ICG 10519	Australia	86	80	83
ICG 10549	Argentina	96	90	93
ICG 10554	Argentina	90	82	86
ICG 10566	Congo	92	94	93
ICG 10595	Brazil	86	80	83
ICG 10616	Argentina	98	92	95
ICG 10788	Tanzania	92	96	94
ICG 10900	Peru	84	98	91
ICG 11130	Brazil	94	74	84
ICG 11203	India	86	90	88
ICG 11605	Bolivia	86	70	78
ICG 12498	Brazil	92	74	83
ICG 12564	Uruguay	92	70	81
ICG 12665	Peru	88	88	88
ICG 12743	Bolivia	92	96	94
ICG 12963	Zimbabwe	86	90	88
ICG 13049	India	90	86	88
ICG 13097	Unknown	96	80	88
ICG 13284	Brazil	92	98	95
ICG 13288	Brazil	98	94	96
ICG 13430	Chad	92	82	87
ICG 13513	Central African Republic	90	98	94
ICG 13829	Uganda	90	78	84
ICG 14007	Central African Republic	82	70	76
ICG 14696	Brazil	90	92	91
var. *hypogaea*				
ICG 956	India	86	72	79
ICG 1975	Sudan	96	74	85
ICG 2422	India	88	70	79
ICG 2506	India	96	76	86
ICG 2777	India	98	73	86
ICG 2925	India	86	71	79
ICG 3877	India	98	76	87
ICG 3987	India	100	76	88
ICG 4243	Australia	94	72	83
ICG 4250	Senegal	88	71	80
ICG 4331	India	96	76	86

Table 1 continued

Identity	Origin	Germination (%)		
		1999/2000 post-rainy	2000/2001 post-rainy	Mean
ICG 4738	United Kingdom	90	76	83
ICG 5163	Brazil	98	78	88
ICG 5233	Israel	90	70	80
ICG 6143	USA	82	76	79
ICG 6361	India	96	75	86
ICG 6515	Israel	100	72	86
ICG 6686	China	86	84	85
ICG 7458	Nigeria	92	82	87
ICG 7932	South Africa	86	71	79
ICG 8748	Russia & CIS	100	82	91
ICG 8833	USA	92	70	81
ICG 8835	USA	94	70	82
ICG 9037	Côte d'Ivoire	98	70	84
ICG 9515	Mozambique	96	74	85
ICG 9556	Mozambique	92	72	82
ICG 9695	India	82	70	76
ICG 9873	Zambia	94	94	94
ICG 10105	Chad	86	82	84
ICG 10575	Israel	94	82	88
ICG 11109	Taiwan	98	80	89
ICG 11456	India	94	76	85
ICG 12360	India	100	83	92
ICG 13539	Togo	98	71	85
ICG 13724	Niger	88	70	79
var. *peruviana*				
ICG 1709	Peru	96	94	95
ICG 1710	Peru	90	96	93
ICG 7293	Peru	94	92	93
ICG 10036	Peru	90	90	90
ICG 10037	Peru	82	100	91
ICG 10567	Peru	92	92	92
ICG 10911	Peru	86	82	84
ICG 10915	Peru	84	84	84
ICG 10945	Peru	88	96	92
ICG 11088	Peru	92	100	96
ICG 12112	Peru	92	94	93
var. *vulgaris*				
ICG 1364	India	86	80	83
ICG 1988	Brazil	96	82	89
ICG 2344	USA	88	78	83
ICG 4749	Argentina	84	82	83

Table 1 continued

Identity	Origin	Germination (%)		
		1999/2000 post-rainy	2000/2001 post-rainy	Mean
ICG 14966	Unknown	92	73	83
Control				
Gangapuri (ICG 2738)	India	84	90	87
ICGS 44 (ICG 13941)	India	43	66	55
ICGS 76 (ICG 13942)	India	50	42	46
M-13 (ICG 156)	India	50	22	36
Trial mean (1,708 entries)		57.9	48.5	53.2
SE±		10.69	7.253	6.20
CV (%)		32.33	18.46	14.5
LSD ($P = 0.05$)		20.95	20.14	17.2

percentage were recorded on a plot basis; number of primary branches, plant height, leaflet length and width, pods per plant, and pod yield per plant were recorded on five competitive plants; seeds per pod, and pod length and width were recorded on ten randomly selected mature pods; seed length and width were based on ten mature seeds; shelling percentage was on 200 g pods; and seed weight was of 100 randomly selected mature seeds. Oven-dried (100°C, 16 h) bulked seed samples were used to determine oil and protein contents in both the seasons. Oil content was determined using a magnetic resonance spectrometer (Jambunathan et al. 1985), and data was corrected to uniform 50 g kg^{-1} seed moisture content. Nitrogen concentration was determined by Technicon Autoanalyser (Pulse Instrumentation Ltd., Saskatoon SK) and then multiplied by 5.46 to convert nitrogen into crude protein content (Singh and Jambunathan 1980).

Data were analyzed by the residual maximum-likelihood (REML) mixed model method with genotypes as random and environments (seasons) as fixed in GENSTAT 9.1 (Payne et al. 2006). The best linear unbiased predictors (BLUPs) were calculated for 15 agronomic and two quality traits. Homogeneity of variances in two seasons was tested by the Bartlett's test of homogeneity (Bartlett 1937). Meta-analysis of two seasons' data was performed when variances were heterogeneous. The components of variance due to the various botanical types as a group and individually and their interactions with season were also estimated for all traits to determine if the botanical types differed or interacted with environment. Also a comprehensive genotype-by-environment analysis, considering all genotypes as one group, was done, and the variance components due to genotype (σ^2_g), genotype-by-environment (σ^2_{ge}), and residual variance (σ^2_e) and their standard errors were calculated.

A phenotypic distance matrix was created by calculating the differences between all pair of accessions using all the descriptors. The diversity index was calculated by averaging all the differences in the phenotypic values for each trait divided by its respective range (Johns et al. 1997).

The mean observations of all traits for each environment were standardized by subtracting from each observation the mean value of the character and dividing by its respective standard deviation, providing standardized values for each trait with an average of 0 and standard deviation of 1. The standardized values were used for principal component analysis (PCA) using GENSTAT 9.1 (Payne et al. 2006). Cluster analysis was performed using scores of the first nine principal components (Ward 1963). Means and variances for quantitative traits in the different clusters were calculated. Differences for means among the clusters were tested using the Newman-Keuls procedure (Newman 1939; Keuls 1952) while the homogeneity of variances among the clusters was tested using Levene's test (Levene 1960).

Results and discussion

Analysis based on botanical varieties

REML analysis indicated that the effect of season was highly significant for all agronomic traits ($P \leq 0.001$–0.005), except for the number of primary branches. The effect of botanical variety was also highly significant ($P \leq 0.001$–0.003) for all traits, except pod and seed width, and 100-seed weight. The season × botanical variety interaction was significant for nine traits ($P \leq 0.001$–0.035), and nonsignificant for six traits (days to flowering, plant height, pod width, seed length, shelling percentage, and seed weight). In the 2001 rainy season, the effect of botanical variety was significant for five traits (leaf and pod length, seed width, plot yield, and shelling percentage) while in the 2001/2002 post-rainy season botanical variety was significant for all traits except for pod and seed width, shelling percentage, and seed weight (data not sown).

Estimates of components of variance for agronomic traits

Genotypic variance (σ^2_g) was significant for 15 traits in 2001 rainy season and for 13 traits in 2001/2002 post rainy-season (Table 2). In the combined analysis (meta analysis) genotypic variance was significant for primary branches, plant height, leaflet length, leaflet width, seed width, and 100-seed weight (Table 2). Genotype-by-environment (σ^2_{ge}) interaction was significant for all 15 traits.

Performance of cold-tolerant germplasm for agronomic traits

Accessions from *fastigiata*, *aequatoriana*, and *peruviana* (subsp. *fastigiata*) groups were compared with the control cultivar Gangapuri; accessions from *vulgaris* (subsp. *fastigiata*) were compared with control cultivar ICGS 44; and those belonging to *hypogaea* (subsp. *hypogaea*) were compared with with control cultivars ICGS 76 and M13. Table 3 lists the accessions with superior performance over their respective controls for various traits among different botanical varieties in the rainy and post-rainy seasons and across seasons. Of these, only 41 accessions from the five botanical varieties were significantly superior to their respective controls for 1–3 traits in the combined analysis. For example, 15 accessions were superior to the controls for pod yield (ICG#10915, 10567, 1710, 11088, 10945, 12625, 7898, 11130, 6148, 6022, 7013, 7905, 7884, 9515, and 4992), 5 for faster seed emergence (ICG#2422, 1364, 2344, 4749, and 1988), 1 for days to 50% flowering (ICG 14966), 9 for oil (ICG# 8833, 9695, 10575, 10036, 11203, 6340, 13513, 13430, and 14007) and 11 for protein (ICG# 9556, 8835, 9515,

Table 2 Estimates of variance components due to genotype (σ^2_g) and genotype × environment (σ^2_{ge}) and their standard errors (SE) for 15 quantitative traits in the 2001 rainy and 2001/2002 post-rainy seasons and combined analysis in cold-tolerant peanut germplasm evaluated at ICRISAT, Patancheru, India

Trait	2001 rainy season		2001–02 post-rainy season		Combined analysis			
	σ^2_g	SE	σ^2_g	SE	σ^2_g	SE	σ^2_{ge}	SE
Time to 50% emergence (days)	0.295	0.060	0.176	0.049	0.025	0.041	0.289	0.056
Time to 50% flowering (days)	8.329	0.970	2.547	0.534	0.046	0.622	6.512	0.854
Primary branch (no.)	0.338	0.050	0.024	0.147	0.019	0.062	0.295	0.077
Plant height (cm)	37.990	4.820	9.742	1.226	4.885	2.313	21.272	2.279
Leaflet length (mm)	31.590	4.260	24.580	3.104	6.620	3.330	30.240	3.920
Leaflet width (mm)	1.705	0.310	2.188	0.328	0.630	0.293	2.173	0.343
Pods per plant (no.)	3.775	0.820	3.710	1.250	<0.001	<0.01	4.850	0.770
Pod length (mm)	15.110	2.120	19.749	2.492	2.470	1.800	16.660	2.260
Pod width (mm)	0.818	0.110	2.340	1.720	<0.001	<0.01	1.733	0.238
Seed length (mm)	1.822	0.260	1.811	0.237	0.215	0.185	1.733	0.238
Seed width (mm)	0.175	0.040	0.192	0.035	0.056	0.025	0.124	0.029
Pod yield per plant (g)	2.592	0.670	10.440	2.070	0.340	0.830	5.390	1.230
Pod yield per plot (kg h^{-1})	29123.000	5135.000	256426.000	35076.000	30170.000	16270.000	155732.000	19871.000
Shelling percentage	15.120	2.590	11.430	1.960	1.740	1.560	11.560	2.070
100 seed weight (g)	22.610	3.570	46.470	6.060	6.580	3.240	26.450	3.840

Table 3 Accessions better or significantly better for various traits compared with their respective control cultivars in the 2001 rainy and 2001-2002 post-rainy seasons and combined analysis

Trait	2001 rainy season	2001–2002 post-rainy season	Combined
Emergence	FST[a]: 1899, 6725, 7005, 7812, 3125, 13097, 115, and 3219[f]	AEQ[e]: 7898	AEQ: 12625
	PRU[b]: 10911	FST: 6221, 5964, 9929, 389, and 4992	FST: 7355, 6725, 115, 4992, 389
	VUL[c]: 1988, 4749 and 2343	HYP: 11456*, 6686*, 13724*, 9556* and 9515*	HYP: 2422*, 6686, 7932, 9556, 7458,
	HYP[d]: 2925*, 6686*, 7458*, 1975*, 2506* and 956*	PRU: 11088, 10567, 1709, 10911 and 7293	PRU: 10567, 1709, 7293, 10911
	VUL: 1988*, 2344, 14966, 4749, 1364	VUL: 1364*, 2344*, 4749*, 1988*	
Days to flowering	FST: 7978*, 4890, 7352, 6340, 10616, 10402,	FST: 1899*, 8570, 7285, 1158, 5964	FST: 7978, 7285, 10402, 1158, 1899
	3125, 115, 3219, 6570, 8514, 12498	HYP: 1975, 6143, 6515	HYP: 6515, 1975, 956, 9556, 6143
	VUL: 1988, 4749, 2344	VUL: 14966, 4749	VUL: 14966*
Pod yield per plot	AEQ : 12625*	AEQ 12553*, 12625*, 7898*, 12719	AEQ: 12625*, 7898*
	FST: 13284*, 2039*, 13513* and 1824*	FST: 10595*, 6148*, 6022*, 7013*, 7884*, 7905* and 4992*	FST: 11130*, 6148*, 7013*, 6022*, 7905*, 7884*, 4992*
	HYP: 6686*	PRU: 10037*, 10567*, 1710*, 11088* and 10945*	HYP: 9515*, 1975, 9556, 956, 13539
	PRU: 10036*, 10567*, 1710*, 1709*, 10915*, 10945*, 11088*		PRU: 10915*, 10567*, 1710*, 11088*, 10945*
Pods per plant	AEQ 12719, 12625	AEQ: 12719, 7898, 12625, 12553	AEQ: 7898, 12719, 12553, 12625
	FST: 4087*, 1824*, 11130* and 8360*	FST: 11130, 10616, 2145, 7929, 5609	FST: 1899, 7929, 13049, 5609, 11130
	HYP: 6686*, 11109, 3877, 4431, 5163, 1975	HYP: 3987, 7932, 13539, 9037	HYP: 9556, 13539, 9037
	PRU: 10036, 1710, 7293, 1709, 10567	PRU: 10036, 10945, 10911, 10915, 11088	PRU: 10036, 10911, 11088, 10915, 10567
Yield per plant (g)	AEQ: 12719, 12625	AEQ: 12625*, 12553*, 7898*	AEQ: 12625*, 7898*, 12719, 12553
	FST: 13513*, 1824*, 13049*	FST: 4992* and 6022*	FST: 6022*, 12963, 13049, 6340, 4992
	HYP: 4331, 1975, 7932, 6686, 11109	HYP: 13539	HYP: 7932, 13539
	PRU: 7293, 10567, 1710, 11088	PRU: 12112*, 1710*, 10915*, 10945*, 10911* and 11088*	PRU: 10911*, 10567*, 12112*, 10915*, 10945*, 1710*, 11088*
	VUL: 2344, 14896		VUL: 2344
Shelling percentage	FST: 13829, 1796, 14696, 1824 and 2159	FST: 12498, 11203, 10549, 397 and 3510	FST: 6570, 12498, 13829, 14696, 11203
	PRU: 1710	HYP: 1975	
		VUL: 10037	
100-Seed weight	AEQ: 12625	AEQ: 12625, 12553, 7898	AEQ: 7898, 12625
	FST: 8485*, 457, 6220, 1274, 1824	FST: 7013*, 14007*, 6022* and 6148*	FST: 8485*, 14007*, 6022*, 6148*
	PRU: 1709, 11088, 12112, 1710	HYP: 8748	PRU: 12112*, 1710*, 10567, 10037, 11088
	VUL: 14966	PRU: 12112* and 1710*	VUL: 14966
			VUL: 14966, 1364

Table 3 continued

Trait	2001 rainy season	2001–2002 post-rainy season	Combined
Oil content[f]	AEQ: 12553, 12719, 12625	AEQ: 7898, 12625, 12553, 12719	AEQ: 12625
	FST: 14007*, 13513*, 13430*	FST: 7777, 282, 10900, 12743, 12665	FST: 11203*, 6340*, 14007*, 13513*, 13430*
	HYP: 10575*, 9695, 8833	HYP: 3987, 2925, 2506, 10575, 4250	HYP: 8833*, 9695*, 10575*, 9873, 6686, 11109
	PRU: 1710*, 12112* and 10036*	PRU: 11088, 10945, 12112, 1710, 10036	PRU: 10036*
		VUL: 4749, 1364	VUL: 1364
Protein content[f]	FST: 1256, 7355, 398, 8485, 1386	FST: 8570, 13430, 1908, 2057, 445	AEQ: 12553
	HYP: 1975*, 10105*, 9515, 4331, 8833	HYP: 4243, 8835, 956, 7932, 6515	FST: 1256*, 7355*, 398*, 8485*, 1384*
	VUL: 1364	VUL: 1364, 14966, 4749, 1988	HYP: 9556*, 8835*, 9515*, 10105*, 4331*, 1975*

[a] FST = *Fastigata*, [b] PRU = *Peruviana*, [c] VUL = *Vulgaris*, [d] HYP = *Hypogaea*, [e] AEQ = *Aequatoriana*, [f] Analysis carried out at entry level only

* Accessions significantly better over their respective controls

10105, 4331, 1256, 1975, 7355, 398, 8485, and 1384) contents, 3 for pod yield and seed weight (ICG#1710, 6022, and 6148), and 1 each for pod yield and protein content (ICG 9515), seed weight and protein content (ICG 8485), and for seed weight and oil content (ICG 14007). However, only ICG# 12625, 10567, 1710, 10945, and 11088 were significantly superior for pod yield in both seasons. The variable performance of many accessions was mainly due to the significant genotype-by-environment interaction observed for most traits (Table 2).

Cluster composition and variation for morphological traits among clusters

PCAs based on the first nine principal components accounted for 79% of the total variation and resulted in four distinct clusters (Table 4). Cluster 1 comprised 23 accessions dominated by *peruviana* (47.8%) and *fastigiata* (30.4%) types. A majority of accessions in this cluster have erect growth habit, sequential branching, no stem pigmentation, leaflets almost glabrous on surfaces, peg pigmentation, pods with slight constriction and reticulation, and had 3-2-4-1/3-2-1-4/3-4-2-1 seeds per pod (more three-seeded than the other type of pods). Although there were five primary seed colors most of the accessions had tan-colored seeds. Of the 26 accessions included in cluster 2, 81% were *hypogaea* types, with most of the accessions having procumbent growth habit, alternate branching, no pigmentation on the stem (but pigmentation on pegs), subglabrous hairs in one or two rows along main axis, and green and glabrous leaflets. Most of the accessions have moderate pod beak and constriction, slight reticulation, and 2-1 seeds per pod (a high frequency of more two-seeded pods). The predominant seed color in this cluster was tan but the cluster included eight primary seed colors. In cluster 3, 54.3% of the accessions belonged to *fastigiata* and 34.3% to *hypogaea*. The cluster was characterized by erect growth with sequential branching, stem and peg pigmentation, subglabrous hairs on the main axis, light green glabrous leaflets, pods with slight beak, constriction and reticulation, and 3-2-4-1/3-2-1-4/3-4-2-1 seeds per pod. Red- and tan-colored seeds were predominant, although nine primary seed colors were recorded. Cluster 4 accessions were predominantly from *fastigiata* (95%), mostly with erect growth habit, sequential branching, stem and peg pigmentation, subglabrous hairs on the main axis, light-green glabrous leaflets, slight pod beak and constriction, and 3-2-4-1/3-2-1-4/3-4-2-1 seeds per pod. Red seed color was predominant, although eight primary seed colors were observed.

Variation for agronomic traits among clusters

In the rainy season the four clusters differed significantly for all traits except for pods per plant and pod

Table 4 Distribution of cold-tolerant peanut accessions and control cultivars in four clusters delineated by cluster analysis based on scores of nine principal components

Cluster	Botanical varieties	Accessions (ICG number)
1	*aequatoriana*	ICGs 7898, 12553, 12625, and 12719
	fastigiata	ICGs 4992, 6022, 6148, 6340, 7013, 7884, and 14007
	hypogaea	ICG 7932
	peruviana	ICGs 1709, 1710, 7293, 10036, 10037, 10567, 10911, 10915, 10945, 11088, and 12112
2	*fastigiata*	ICGs 1683, 10075, 10554, and 11203
	hypogaea	ICGs 956, 1975, 2422, 2506, 2777, 2925, 3877, 3987, 4331, 5163, 6143, 6361, 8748, 8833, 8835, 9037, 9556, 9695, 10105, 10575, and 11109
	vulgaris	ICG 2344
3	*fastigiata*	ICGs 457, 1274, 1796, 1824, 2158, 2159, 3510, 4670, 4890, 5609, 5964, 6220, 6565, 8485, 9141, 9144, 9929, 10481, and 10595
	hypogaea	M 13, ICGs 4243, 4250, 4738, 6686, 9515, 9873, 11456, 12360, 13539, and 13724, and ICGS 76
	vulgaris	ICGs 1364, 4749, 14966 and ICGS 44
4	*fastigiata*	ICGs 115, 282, 318, 376, 389, 397, 398, 445, 1158, 1256, 1298, 1384, 1399, 1899, 1908, 2039, 2057, 2145, 3125, 3219, 3477, 3726, 3779, 4087, 4788, 5094, 5475, 6203, 6221, 6421, 6570, 6706, 6725, 6878, 6888, 7005, 7285, 7352, 7355, 7777, 7812, 7905, 7929, 7978, 8360, 8514, 8517, 8570, 10092, 10371, 10402, 10495, 10519, 10549, 10566, 10616, 10788, 10900, 11130, 11605, 12498, 12564, 12665, 12743, 12963, 13049, 13097, 13284, 13288, 13430, 13513, 13829, and 14696, and Gangapuri
	hypogaea	5233, 6515, and 7458
	vulgaris	1988

width (Table 5). Cluster 2 and 4 accessions emerged faster and flowered earlier. Accessions in cluster 4 were taller, had large leaflets, and high seed protein content. Accessions in cluster 3 were shorter and had small leaflets, more primary branches, the highest seed yield, a high shelling percentage, and large seed size. Accessions in cluster 2 had the highest seed oil content. In the post-rainy season, differences were significant among four clusters for 17 traits. Cluster 1 and 4 accessions emerged faster and flowered earlier. Plants in cluster 1 accessions were taller, had larger leaflets, highest seed yield, large pod and seed size, and high seed oil content.

Heterogeneity of variances for various traits

Variance for 9 agronomic traits in the 2001 rainy and 8 traits in the 2001/2002 post-rainy seasons were heterogeneous (Table 6). In the rainy season, cluster 1 accessions had higher variances for plant height and leaflet length and cluster 3 accessions for pod yield per plot, 100-seed weight, and seed protein content. Higher variances for accessions in cluster 1 for pod length, in cluster 2 for pod yield and 100-seed weight, and in cluster 3 for pods per plant occurred in the post-rainy season.

Phenotypic diversity index among clusters

The clusters differed in terms of the biological status (whether landraces, breeding lines or cultivars) of the accessions involved in minimum and maximum phenotypic diversity indices (Table 7). Accessions with the least difference in phenotypic diversity index in the entire set were ICG 13288 (a landrace from Brazil) and ICG 10519 (an advanced cultivar from Australia). The accessions with the minimum diversity index in each cluster were two landraces from Peru (ICG 10945 and ICG 11088) in cluster 1, breeding lines (ICG 2506 and ICG 2925) from India in cluster 2, a landrace (ICG 6220) from Brazil and an advanced cultivar (ICG 9144) from Syria in cluster 3, and an accession with unknown origin (ICG 1298) and Indian cultivar Gangapuri (ICG 2738) in cluster 4.

The maximum phenotypic diversity index in the entire set was between ICG 12112 (landrace from

Table 5 Mean for agronomic traits in different clusters of cold-tolerant peanut germplasm accessions in the 2001 rainy and 2001–2002 post-rainy seasons at ICRISAT Center, Patancheru, India

Trait	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Rainy season				
Time to emergence (days)	8.0b	8.0b	8.4a	8.4a
Time to 50% flowering (days)	21.1b	19.8c	25.4a	19.9c
Primary branch (no.)	4.6b	4.4b	5.3a	4.4b
Plant height (cm)	28.6a	28.8a	18.7b	30.6a
Leaflet length (mm)	54.2a	53.5a	44.7b	55.2a
Leaflet width (mm)	23.0a	22.9a	21.5b	23.2a
Pods per plant (no.)	10.1a	10.7a	10.3a	10.7a
Pod length (mm)	33.3a	33.0a	31.0b	33.5a
Pod width (mm)	12.8a	12.8a	12.5a	12.8a
Seed length (mm)	14.2ba	13.9b	14.7a	14.0ba
Seed width (mm)	7.7b	7.6b	7.9a	7.6b
Pod yield per plant (g)	7.8a	7.9a	7.1b	7.8a
Pod yield per plot (kg)	529.2b	517.2b	617.9a	492.8b
Shelling (%)	58.1b	59.8ba	61.1a	59.0b
100-seed weight (g)	32.6b	32.1b	35.0a	32.3b
Oil content (%)	47.7ba	48.3a	47.1bc	46.5c
Protein content (%)	25.3b	23.7c	25.5b	27.4a
Post-rainy season				
Time to emergence (days)	12.0c	12.8a	12.4b	12.1c
Time to 50% flowering (days)	48.3b	50.0a	48.0b	47.6b
Primary branch (no.)	4.5b	4.7a	4.6b	4.5b
Plant height (cm)	22.1a	13.0d	16.5c	18.1b
Leaflet length (mm)	57.5a	41.9c	46.0b	47.4b
Leaflet width (mm)	24.3a	19.8c	20.7b	21.2b
Pods per plant (no.)	16.2b	17.3a	16.0b	15.0c
Pod length (mm)	40.2a	33.3b	34.6b	35.2b
Pod width (mm)	14.3a	13.6b	13.8b	13.8b
Seed length (mm)	14.5a	13.7b	13.5b	12.7c
Seed width (mm)	8.3a	8.0b	8.3a	8.2a
Pod yield per plant (g)	22.3a	17.8b	17.5b	15.4c
Pod yield per plot (kg)	2562.7a	1745.4b	1688.4b	1360.2c
Shelling (%)	65.8b	66.1b	68.0a	68.4a
100-seed weight (g)	53.9a	44.3c	48.0b	46.7cb
Oil content (%)	49.6a	48.9ba	47.8ba	47.4c
Protein content (%)	23.6b	23.2b	24.6ba	25.3a

Differences between means of different clusters were tested using the Newman–Keuls test

Means followed by the same letter are not significantly different at $P = 0.05$

Peru) and ICG 156 (cultivar from India) while accessions with maximum diversity in individual clusters were ICG 12112 (landrace from Peru) and ICG 7932 (advance line from South Africa) in cluster 1, ICG 10075 (landrace from Peru) and ICG 9037 (landrace from Côte d'Ivoire) in cluster 2, ICG 10595 (landrace from Brazil) and ICG 13942 (cultivar from India) in cluster 3, and ICG 1908 (breeding line from India) and ICG 7352 (land race from Peru) in cluster 4.

Table 6 Traits with heterogeneous variances in four clusters for various agronomic traits in cold-tolerant peanut germplasm accessions, 2001-rainy and 2001–2002 post-rainy seasons, at ICRISAT, Patancheru, India

Trait	Entire	Cluster 1	Cluster 2	Cluster 3	Cluster 4	*F* value	*P*[a]
Rainy season							
Time to 50% emergence (days)	0.2	0.2	0.1	0.2	0.1	4.48	0.0048
Primary branch (no.)	0.2	0.2	0.1	0.2	0.1	3.99	0.0089
Plant height (cm)	34.5	27.4	14.5	11.4	8.7	3.93	0.0098
Leaflet length (mm)	28.9	26.3	7.5	7.7	10.8	4.65	0.0038
Leaflet width (mm)	1.2	1.3	0.5	0.2	0.9	4.74	0.0034
Pods per plant (no.)	2.2	1.6	1	3.1	2.4	2.75	0.0449
Pod yield per plot(kg)	22725.9	23830.5	11155.8	43158	13063.4	4.55	0.0043
100-seed weight (g)	17.3	16.3	11.6	37.9	8.3	10.25	<.0001
Protein content (%)	5.6	2.6	5.7	6.8	2	5.37	0.0015
Post-rainy season							
Pods per plant (no.)	4.1	4.3	4	5.4	2.1	3.37	0.0200
Pod length (mm)	18.9	39.9	24.1	9.4	7.4	7.39	0.0001
Seed length (mm)	1.8	2.2	3.1	1.6	0.5	8.87	<.0001
Seed width (mm)	0.1	0.1	0.1	0.1	0.1	2.96	0.0341
Pod yield per plant (g)	14.1	10.6	15.2	15	3.9	4.98	0.0025
Pod yield per plot (kg)	390860.7	334815.9	401850.4	392666.8	80495.8	5.54	0.0012
Shelling (%)	6.8	5.5	14.5	5.7	3	7.56	<.0001
100-seed weight (g)	37.1	61	66.3	26	10.7	6.63	0.0003

Variances were tested using Levene's test

[a] P = probability at 0.5

Table 7 Phenotypic diversity in the cold-tolerant peanut germplasm accession in the entire set and in different clusters

Entire set	Phenotypic diversity
Mean phenotypic diversity index	0.188
Minimum phenotypic diversity index between ICG10519 and ICG 13288	0.062
Maximum phenotypic diversity index between ICG 12112 and ICG 156	0.408
Cluster 1	
Mean phenotypic diversity index	0.277
Minimum phenotypic diversity index between ICG 10945 and ICG 11088	0.145
Maximum phenotypic diversity index between ICG 12112 and ICG 7932	0.437
Cluster 2	
Mean phenotypic diversity index	0.261
Minimum phenotypic diversity index between ICG 2506 and ICG 2925	0.138
Maximum phenotypic diversity index between ICG 10075 and ICG 9037	0.517
Cluster 3	
Mean phenotypic diversity index	0.255
Minimum phenotypic diversity index between ICG 6220 and ICG 9144	0.112
Maximum phenotypic diversity index between ICG 10595 and ICG 13942	0.450
Cluster 4	
Mean phenotypic diversity index	0.209
Minimum phenotypic diversity index ICG 1298 and ICG 2738	0.101
Maximum phenotypic diversity index between ICG 1908 and ICG 7352	0.410

Botanical varieties and cold tolerance

Differences for chilling injury among botanical types of peanut have been reported. Sellschop and Salmon (1928) found Valencia and Spanish types highly sensitive while Virginia bunch type had exceptional hardiness. Bell et al. (1991) reported a positive association between rate of emergence and mean daily temperature (17.8–23.2°C) in 16 peanut cultivars, indicating that air temperatures were always lower than those required for good germination. They however reported no significant differences ($P < 0.05$) in coefficients of temperature sensitivity either between cultivars of the same botanical type or between different botanical types. All cultivars used in their study had similar base temperature (T_b) values for emergence (13.2°C). However, we found differences between accessions (irrespective of botanical type) in terms of their cold tolerance at emergence under lower temperatures (12°C) under laboratory conditions (Upadhyaya et al. 2001), indicating that cold-tolerant accessions identified in this study captured greater diversity for base-temperature tolerance at germination.

The cold-tolerant accessions reported in this study had substantial diversity for most agronomic traits and thus should be good sources to use in breeding programs for developing peanut cultivar that germinate at lower temperatures. It will also be interesting to study the reaction of these cold-tolerant accessions at various growth stages at which peanut is vulnerable to cold injury. Some of the identified accessions have good agronomic potential, and hence their use in breeding programs will not adversely affect exploitation of additive genetic variance in a self-pollinated crop such as peanut.

References

Agriculture Canada (1984) Modified description of variety. Peanut (*Arachis hypogaea* L.), variety OAC Garroy. Licensed no. 2439

Agriculture Canada (1989) Description of variety. Peanut (*Arachis hypogaea*, L.), variety OAC Ruby. Registration no. 1–82. Agriculture Canada, Ottawa

Bartlett MS (1937) Some examples of statistical methods of research in agriculture and applied biology. J R Stat Soc 4:137–170

Bell MJ, Shorter R, Mayer R (1991) Cultivar and environmental effects on growth and development of peanuts (*Arachis hypogaea* L). 1. Emergence and flowering. Field Crops Res 27:17–33

Bell MJ, Roy RC, Tollenaar M, Michaels TE (1994a) Importance of variation in chilling tolerance for peanut genotypic adaptation to cool short-season environments. Crop Sci 34:1030–1039

Bell MJ, Gillespie TJ, Roy RC, Michaels TE, Tollenaar M (1994b) Peanut leaf photosynthetic activity in cool field environments. Crop Sci 34:1023–1029

Benedict CR, Ketring DL (1972) Nuclear gene affecting greening in virescent peanut leaves. Plant Physiol 49:974–976

Bhagat NR, Ahmad T, Lalwani HB, Patra GJ, Acharya D (1988) Screening of bunch peanut (*Arachis hypogaea* L. ssp. *fastigiata*) germplasm for cold tolerance in India. Trop Agric (Trinidad) 65:109–112

Bhagat NR, Dayal D, Acharya D (1992) Performance of Spanish peanuts during winter- summer season at two locations in India. Trop Agric (Trinidad) 69:93–95

Bunting AH, Gibbons RW, Wynne JC (1985) Peanut (*Arachis hypogaea* L.). In: Summerfield RJ, Roberts EH (eds) Grain legume crops. Collins, London, pp 747–800

Dan NT, Hong NX (1996) Collaborative research in Vietnam: needs and opportunities. In Gowda CLL, Ramakrishna A (eds) Summary proceedings of CLAN country coordinators' steering committee meeting, 4–6 Dec. 1995, ICRISAT Patancheru, India, pp 56–58

Ellis RS, Hong TD, Roberts EH (1985) The conduct of seed germination tests. Handbook of seed technology for genebanks. Vol. 1. Principles and methodology. IBPGR Rome, Italy, pp 94–198

Fu JR, Lu XH, Chen RZ, Zang BZ, Liu ZS, Lee ZS, Cai DY (1988) Osmoconditioning of peanut (*Arachis hypogaea* L.) seeds with PEG to improve vigour and some biochemical activities. Seed Sci Technol (Switzerland) 16:197–212

Golombok SD, Johanson C (1997) Effect of soil temperature on vegetative and reproductive growth and development in three Spanish genotypes of peanut (*Arachis hypogaea*). Peanut Sci 24:67–72

IBPGR and ICRISAT (1992) Descriptors for groundnut. International Board for Plant Genetic Resources, Rome, Italy; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, 125 pp

Jambunathan R, Raju SM, Barde P (1985) Analysis of oil contents of groundnut by nuclear magnetic resonance spectrometry. J Sci Food Agric 36:162–166

Johns MA, Skroach PW, Nienhuis J, Hinrichsen P, Bascur G, Munoz-Schick C (1997) Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. Crop Sci 37:605–613

Ketring DL (1984) Temperature effects on vegetative and reproductive development of peanut. Crop Sci 24:877–882

Ketring DL, Brown RH, Sullivan GA, Johnson BB (1982) Growth physiology. In: Pattee HE, Young CT (eds)

Peanut science and technology. Yoakum, Texas, pp 411–457

Keuls M (1952) The use of the 'Studentized range' in connection with an analysis of variance. Euphytica 1:112–122

Koirala GP (1996) Collaborative research in Nepal: Needs and opportunities. In Gowda CLL, Ramakrishna A (eds) Summary proceedings of CLAN country coordinators' Steering committee meeting, ICRISAT, Patancheru, India, pp 39–43

Levene H (1960) Robust tests for equality of variances. In: Olkin I (ed) Contributions to probability and statistics: essays in honour of Harold Hotelling. Stanford University Press, Stanford, pp 278–292

Newman D (1939) The distribution of range in samples from a normal population expressed in terms of an independent estimate of standard deviation. Biometrika 31:20–30

Paterson HD, Williams ER (1976) A new class of resolvable incomplete block designs. Biometrika 63:395–400

Payne RW, Harding SA, Murray DA, Soutar DM, Baird DB, Welham SJ, Kane AF, Gilmour AR, Thompson R, Webster R, Wilson GT (2006) The guide to Genstat release 9, Part 2: statistics. VSN International, Hemel Hempstead

Sellschop JPF, Salmon SC (1928) The influence of chilling above the freezing point on certain crop plants. J Agric Res 37:315–338

Sinclair TR, Bennett JM, Boote KJ (1993) Leaf nitrogen content, photosynthesis and radiation use efficiency in peanut. Peanut Sci 20:40–43

Singh U, Jambunathan R (1980) Evaluation of rapid method for the estimation of protein content in chickpea (*Cicer arietinum* L.). J Sci Food Agric 31:247–254

Singleton JA, Pattee HE (1989) Effect of chilling injury on windrowed peanuts. Peanut Sci 16:51–54

Upadhyaya HD, Nigam SN, Singh S (2001) Evaluation of groundnut core collections to identify sources of tolerance to low temperature at germination. Indian J Plant Genet Resour 14:165–167

Upadhyaya HD, Ortiz R, Bramel PJ, Singh S (2003) Development of a core collection using taxonomical, geographical and morphological descriptors. Genetic Res Crop Evol 50:139–148

Ward J (1963) Hierarchical grouping to optimize an objective function. J Am Stat Assoc 38:236–244