



Choice of Direction of Biparental Crosses to Maximize Genetic Variability in Segregating Populations in Cowpea [*Vigna unguiculata* (L.) Walp]

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ABSTRACT

Background: Segregating populations generated through deliberate hybridization serve as the primary source for developing new pure-line cultivars in predominantly self-pollinated crops, including cowpea. Biparental crosses are commonly used to generate segregating populations, the number of parents involved may differ. The choice of parental direction specifically, which parent is designated as male or female plays a crucial role in optimizing the expression of desirable genetic variability.

Methods: Three sets of F_2 populations were field-evaluated during Rabi 2023-24 at the experimental plots, University of Agricultural Sciences (UAS), GKVK, Bengaluru for pod length (cm), pods plant⁻¹, grain yield plant⁻¹ (g) and 100 grain weight(g). Significance or otherwise of differences among the three F_2 populations were examined using two-sample 't' test with unequal variance. The comparison was based on trait mean, standardized range, variance and usefulness index (UI), which served as the criteria for interpreting significant reciprocal cross differences.

Result: No significant reciprocal effects were observed for most of the traits in the F_2 populations. This indicates that either parent can be used as the female or male in crosses without affecting grain yield performance. However, evaluating reciprocal effects can still inform crossing strategies and potentially enhance breeding efficiency.

Key words: Grain yield, Reciprocal crosses, Transgressive segregation index, Usefulness index.

INTRODUCTION

Cowpea is a self-pollinating annual diploid with chromosomes $2n = 2x = 22$ belong to the Fabaceae family. It is one of the improved legume extensively grown in India and Africa (Boukar *et al.*, 2020). Asia's top cowpea-growing countries include India, Bangladesh, Myanmar, Sri Lanka, China, Korea and Thailand. In India, cowpea is grown across various states, including Karnataka, which is one of the notable contributors to its cultivation. In Karnataka, the crop is cultivated over an area of 78,446 hectares, producing a total output of 35,759 tonnes, with an average productivity of 0.46 t/ha (Indiastat, 2022). It is resilient to tolerate a wide range of environmental stressors, making it the one of the most preferred crops for small-scale farmers. Considering that production of crops including cowpea in highly vulnerable small holding rainfed ecosystem is challenging, development and deployment of high yielding cowpea cultivars resilient to biotic and abiotic stresses with specific adaptation is critical for sustainable cowpea production by resource poor farms (Reddy *et al.*, 2025). Cowpea not only has a limited genetic base but is also experiencing genetic erosion, posing additional challenges to conventional breeding efforts (Tripathi *et al.*, 2019; Horn and Shimelis, 2020). Limits to our understanding of the type of genetic architecture underlying trait differentiation also include the relative contribution of cytoplasmic effects. Geneticists and breeders have recognized reciprocal effects as one source of genetic variability (Mann and Pollmer, 1981).

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Segregating populations derived from planned crosses are most widely used sources of new pure line cultivars in predominantly self-pollinated crops with no exception of cowpea. Biparental crosses are most widely used for deriving segregating populations. Direction of crosses in terms of which of the two parents to be used as male/female is critical in maximizing useful genetic variability. We hypothesized

that there exist significant differences in segregating populations derived from reciprocal crosses. To test this hypothesis, the objective of our study was to examine a possible influence of reciprocal cross effects on grain yield and some quantitative traits in cowpea.

MATERIALS AND METHODS

The material consisted of four genotypes [IC488271, IC263015, IC608044, IC590843] with manifested substantial variability for quantitative traits (Table 1). These four genotypes were used to effect three sets of reciprocal crosses. Four parental genotypes were used to generate three pairs of reciprocal crosses were affected namely C1=IC488271×IC590843, C2=IC590843×IC488271, C3=IC263015×IC590843, C4=IC590843×IC263015, C5=IC608044×IC590843 and C6=IC590843×IC608044 during early *kharif* 2023. IC590843 was employed as the common parent in all crosses owing to its green bold seed size, which is a desirable trait for enhancing grain yield and market acceptability in cowpea. The F_1 's of three pairs of crosses were raised and hybridity was confirmed using male parent-specific traits in each cross like seed size and seed coat color. The true F_1 's of six crosses was selfed. The self-seeds were planted in subsequent season to raise three sets of F_2 populations during *Rabi* 2023-24 at the Experimental plots, University of Agricultural Sciences (UAS), GKVK, Bengaluru (13°05'16.4"N 77°33'55.2"E). After 15 days of planting, seedlings were thinned to maintain 0.2 m between plants within a row. All the recommended agronomic practices were adopted to raise a good crop of cowpeas. The size of three sets of F_2 populations ranged from 101 to 137 (Table 2). The data were recorded on 5 randomly selected plants of parents and all the F_2 plants of six crosses on pod length (cm), pods plant⁻¹, grain yield plant⁻¹ (g) and 100 grain weight (g).

Data analysis

Data recorded on individual F_2 plants were used to estimate descriptive first-degree statistics such as mean, absolute range (AR), standardized range (SR) and second-degree statistics such as absolute phenotypic variance (σ_p^2), phenotypic standard deviation (σ_p) and usefulness index using the following formulae. All the analysis were implemented in 'R' statistical software, version 3.5.2 (R Core Team 2024). The distribution of F_2 populations were graphically plotted using R software.

Arithmetic mean

$$\bar{X} = \frac{\text{Some of observations of ten plants (parents and } F_1\text{'s) or all plants in each of the } F_2 \text{ population}}{\text{Number of plants}}$$

Standardized range (SR) was estimated as

$$\text{Standardized range} = \frac{\text{Highest value} - \text{Lowest value}}{\text{Genral mean } (\bar{X}) \text{ of the respective trait}}$$

The significance of trait means between the three sets of reciprocal crosses (F_2) were examined using two-sample 't' test.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Where,

X_1 = Mean of direct cross.

X_2 = Mean of reciprocal cross.

n_1 = Number of plants in direct cross.

s_1 = Standard deviation of direct cross.

s_2 = Standard deviation of reciprocal cross.

n_2 = Number of plants in reciprocal cross.

Transgressive segregation index (TSI)

TSI was estimated as the proportion of the difference between the parents and the corresponding range for a phenotype in the segregating (F_2) populations. Based on descriptive statistics, TSI was estimated (Koide *et al.*, 2019) as:

$$TSI = \frac{\text{Absolute range in } F_2 \text{ population}}{\text{Trait mean difference between parents}}$$

Levene's test for homogeneity of variances

Levene's test (Levene, 1960) was used to test if σ_p^2 of reciprocal F_2 populations were comparable.

Usefulness index (UI)

UI accounts for trait mean, phenotypic variability and broad-sense heritability and thus provide a more comprehensive and informative statistic in terms of genetic gain expected when different intensities of selection were imposed in segregating populations (Allier *et al.*, 2019; Lehermeier *et al.*, 2017; Bernardo, 2020).

$$Uc = \text{Trait mean} + (k \times \sigma_g^2 / \sigma_p^2)$$

Where,

k = Standardized selection differential at different selection intensities.

k = 2.67 and 2.06 at 1 and 5 per cent selection intensities, respectively (Bernardo, 2020).

σ_g^2 = Genotypic variance.

σ_p^2 = Phenotypic standard deviation.

The ' σ_g^2 ' was estimated as the square root of σ_g^2 ; The σ_g^2 was estimated at $\sigma_p^2 - \sigma_e^2$, where, σ_e^2 for F_2 populations was estimated as the average of phenotypic variance in non-segregating populations (F_1 , P_1 , P_2).

$$\sigma_e^2 = \frac{2VF_1 + VP_1 + VP_2}{4}$$

Criteria to assess the reciprocal cross differences in F_2 segregating populations

Significant trait means, standardized range, variance and UI between reciprocal F_2 populations derived from three crosses were interpreted as significant reciprocal cross differences.

RESULTS AND DISCUSSION

The success of plant breeding hinges on the level of genetic variability within the working germplasm and/or in the segregating generations derived from carefully chosen parents. Although biparental crosses are commonly employed to develop segregating populations, the number of parents involved can vary. We hypothesize that even the direction of both bi- and multiparent crosses could markedly influence the resulting genetic variability. In this manuscript, we refer to segregating populations as breeding populations (BPs). From a plant breeding perspective, the most promising BP is one that shows both a high mean for the target trait and substantial genetic variance. A higher mean ensures a head start, while greater variance provides scope for effective selection. In this background, the findings of the present investigation are discussed.

It is evident from the results that straight and reciprocal crosses were comparable for means of all the four traits, though trait means *per se* varied with the cross combinations (C1/C2, C3/C4 and C5/C6). Further, means of all the four traits of cross combination namely C5/C6 were significantly greater than the other two cross combinations (Fig 1). These results suggest that there were no significant reciprocal cross differences for trait means. These findings are consistent with those reported by Kovačević *et al.* (2022) in maize, where the maternal inbred line did not exhibit statistically significant differences between the two variants, indicating a minimal reciprocal effect.

As is true with respect to trait means, estimates of standardized range (SR) of all the four traits were also comparable between straight and reciprocal crosses of all the cross combinations (C1/C2, C3/C4 and C5/C6). However contrary to trait mean, estimates of SR of the cross combination namely C5/C6 was significantly lower than other two cross combinations (Table 3). Considering that without transgressive segregation (TS), plant breeding does not work; plant breeding does work and because TS occurs (Mackay *et al.*, 2020). The estimates of TSI were relatively close between members of each reciprocal cross pair (C1/C2, C3/C4 and C5/C6), indicating comparable TS for all the traits. While C1/C2 displayed higher TSI than other crosses for grain yield, C3/C4 exhibited higher TSI for pods plant⁻¹; C5/C6 showed higher TSI for pod length and 100 grain weight (Table 3). Theoretical investigations have indicated that TS results from dispersion of favourable complementary alleles between the parents from which BPs are derived (Bernardo, 2020; Mackay *et al.*, 2020). These theoretical studies suggests that alleles that increase grain weight plant⁻¹ are dispersed between parents (IC488271 and IC590843) of C1/C2 cross, while those that increase pods plant⁻¹ are dispersed between parents (IC263015 and IC590843) of C3/C4 cross; those that increase pods length and 100 grain weight are dispersed between parents (IC608044 and IC590843) of C5/C6 cross.

Quantifying the variability for target traits is critical in addition to means to make a better-informed choice about

Table 1: Details of parental genotypes with accession numbers.

Accession number	Pod length (cm)	Pods per plant	Grain yield per plant (g)	100 grain weight (g)
IC488271	6.80	22	9.55	4.34
IC263015	11.00	17	16.71	7.70
IC608044	17.00	20	20.34	18.03
IC590843	18.50	15	10.50	20.80

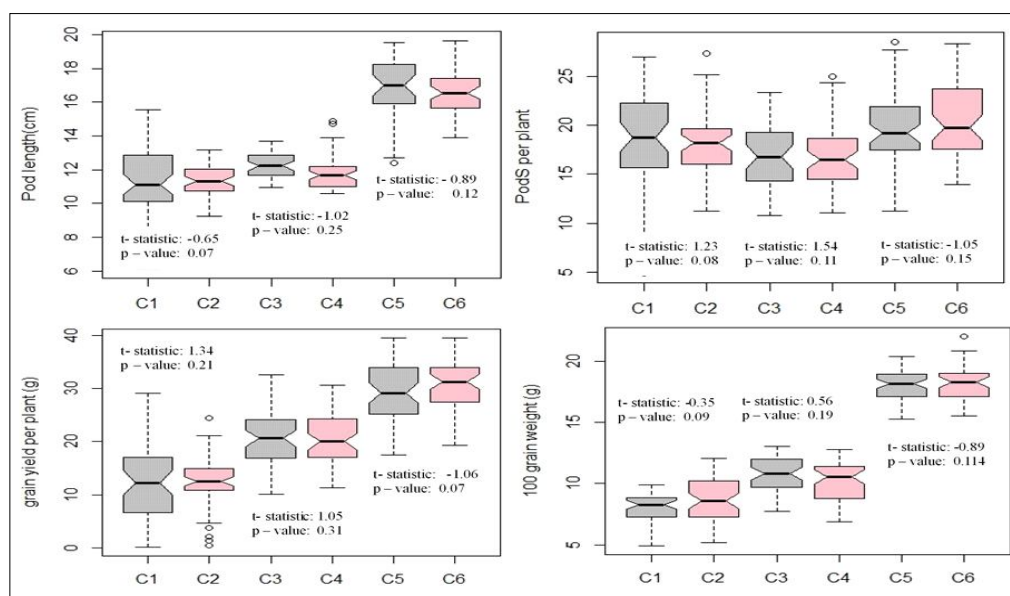


Fig 1: Box-whisker plot depicting statistical difference between three pairs of F₂ populations for four quantitative traits in cowpea.

the selection of BPs (Vaggar *et al.*, 2022). Crop breeders target highly variable segregating populations as they offer ample opportunities for selection and genetic improvement (Ongom *et al.*, 2021). The estimates of absolute phenotypic variance of all the traits were comparable between all the cross combinations (Table 4). Further, C5/C6 were more

variable than C1/C2 and C3/C4 for pods plant⁻¹ and grain yield plant⁻¹. These results are comparable to those observed in maize (Pollmer *et al.*, 1979; Arellano-Vázquez *et al.*, 2023). However, the proportion of genetic variance attributable to reciprocal differences was relatively small (Melchinger *et al.*, 1985; Carena, 2005; Easterly *et al.*, 2020; Demail *et al.*, 2023).

The estimates of UI were also comparable between straight and reciprocal cross combinations at 1% and 5% selection intensities (Fig 2). From the aforesaid discussion, it is evident that best BPs differed with the criteria. For example, based on SR and variance, C1/C2 and C3/C4 were better than the other cross. On the other hand, the cross C5/C6 was better than the other two cross combination based on mean and UI. Thus, the cross combination C5/C6 could be regarded as promising as

Table 2: Details of cross identity with size of F₂ population.

Identity of cross	Crosses	Size of F ₂
C1	IC488271×IC590843	115
C2	IC590843×IC488271	120
C3	IC263015×IC590843	101
C4	IC590843×IC263015	118
C5	IC608044×IC590843	125
C6	IC590843×IC608044	137

Table 3: Estimates of SR and TSI among F₂ derived from sets of reciprocal crosses in cowpea.

Traits	SR						TSI					
	C1	C2	C3	C4	C5	C6	C1	C2	C3	C4	C5	C6
Pod length (cm)	0.94	0.99	0.80	0.81	0.35	0.38	0.90	0.93	1.28	1.31	3.79	4.15
Pods per plant	2.26	2.94	2.53	2.75	2.95	2.62	6.14	7.57	21.50	22.00	11.80	11.00
Grain yield per plant (g)	3.75	4.45	1.99	2.33	2.05	2.29	47.61	53.80	6.48	7.36	6.12	7.04
100 grain weight(g)	1.18	1.14	1.04	1.03	0.74	0.68	0.59	0.58	0.84	0.80	4.83	4.43

Table 4: Estimates of absolute phenotypic variance and Levene- test statistics among F₂ derived from sets of reciprocal crosses in cowpea.

Traits	C1	C2	Levene- test statistic	C3	C4	Levene- test statistic	C5	C6	Levene- test statistic
Pod length (cm)	3.41	3.22	0.42 ^{NS}	3.69	4.01	0.35 ^{NS}	3.11	3.25	0.62 ^{NS}
Pods per plant	45.62	50.62	0.756 ^{NS}	38.52	40.65	0.85 ^{NS}	51.24	54.65	0.95 ^{NS}
Grain yield per plant (g)	56.45	60.25	0.86 ^{NS}	58.23	62.58	0.96 ^{NS}	64.25	68.56	0.99 ^{NS}
100 grain weight (g)	5.23	6.45	0.53 ^{NS}	4.56	4.89	0.63 ^{NS}	4.11	4.89	0.54 ^{NS}

^{NS} denotes non-significant difference between pair of crosses.

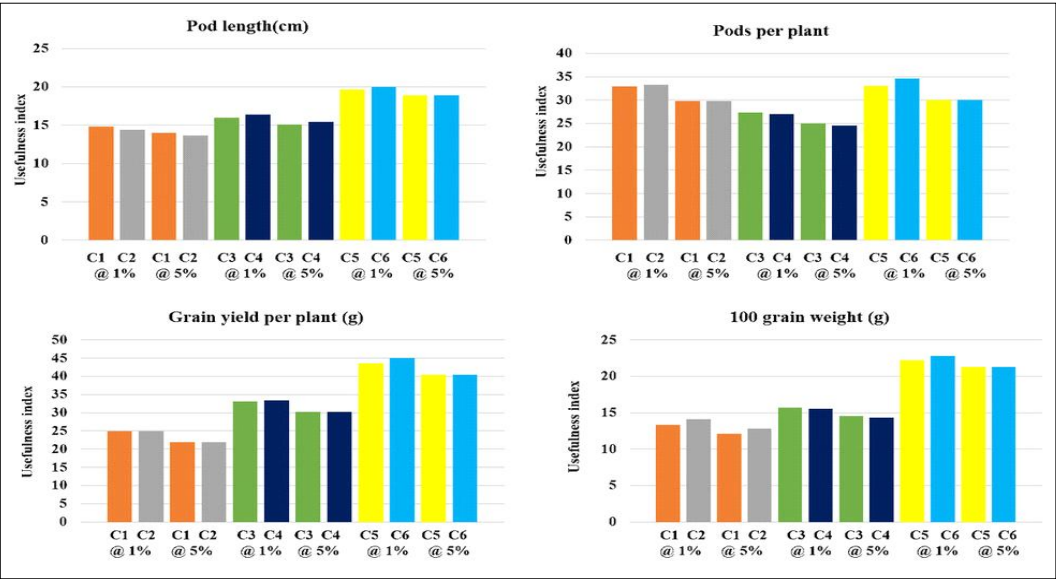


Fig 2: Bargraphs showing differences in estimates of usefulness criterion for grain yield and its component traits at different selection differential (k) in three F₂ pairs of cowpea.

per as probability of deriving pure-lines better than their parents is concerned. Further, it appears that direction of the cross is not significant for deriving BPs. Thus, the present results did not support our hypothesis of significant differences between reciprocal crosses.

CONCLUSION

Reciprocal crosses between the populations created hybrid seed with the same nuclear genetic composition, but different cytoplasmic genes. The genetic analysis of F_2 pairs showed no statistically significant reciprocal effects for key grain yield traits. This suggests that either direction of the cross could be used to generate variability for grain yield traits and any parental line can serve as either the maternal or paternal component in a cross combination. For that reason, breeders should take the reciprocal effects in consideration in planning crossings strategies. This knowledge might be useful in deciding about the sequence of parents to exploit the reciprocal differences to an advantage. However, if no significant reciprocal differences are detected in cowpea, hybridization programmes should instead emphasize other critical factors such as the genetic divergence between parents, combining ability for yield and adaptive traits, heterosis potential, heritability estimates and stability of performance across environments.

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Conflict of interest

The authors declare that they have no conflict of interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

- Allier, A., Moreau, L., Charcosset, A., Teyssedre, S., Lehermeier, C. (2019). Usefulness criterion and post-selection parental contributions in multiparental crosses: Application to polygenic trait introgression. *G3 Genes Genom Genet.* **9**: 1469-1479.
- Arellano-Vázquez, J.L., Gutiérrez-Hernández, G.F., Ceja-Torres, L.F., Flores-Gómez, E., García-Ramírez, E., Quiroz-Figueroa, F.R. and Vázquez-Lozano, P. (2023). Combining ability and reciprocal effects for the yield of elite blue corn lines from the Central Highlands of Mexico. *Plants.* **12**(22): 3861.
- Bernardo, R. (2020). Breeding for Quantitative Traits in Plants. 3rd Edn, Stemma Press, Woodbury, Minnesota, USA.
- Boukar, O., Abberton, M., Oyatomi, O., Togola, A., Tripathi, L., Fatokun, C. (2020). Introgression breeding in cowpea [*Vigna unguiculata* (L.) Walp.]. *Front Plant Sci.* **11**: 567425.
- Carena, M.J. (2005). Maize commercial hybrids compared to improved population hybrids for grain yield and agronomic performance. *Euphytica.* **141**: 201-208.
- Dermail, A., Lübberstedt, T., Suwarno, W.B., Chankaew, S., Lertrat, K., Ruanjaichon, V. and Suriharn, K. (2023). Combining ability of tropical × temperate maize inducers for haploid induction rate, R1-nj seed set and agronomic traits. *Front. Plant Sci.* **14**: 1154905.
- Easterly, A.C., Garst, N., Belamkar, V., Ibrahim, A.M., Rudd, J.C., Sarazin, J.B. and Baenziger, P.S. (2020). Evaluation of hybrid wheat yield in Nebraska. *Crop Sci.* **60**(3): 1210-1222.
- Horn, L.N., Shimelis, H. (2020). Production constraints and breeding approaches for cowpea improvement for drought prone agro-ecologies in Sub-Saharan Africa. *Ann Agric Sci.* **65**(1): 83-91.
- Indiastat, (2022). Cowpea Area, Production and Productivity in Karnataka. Indiastat Database. Available at: <https://www.indiastat.com>.
- Koide, Y., Sakaguchi, S., Uchiyama, T., Ota, Y., Tezuka, A., Nagano, A.J., Ishiguro, S., Takamura, I. and Kishima, Y. (2019). Genetic properties responsible for the transgressive segregation of days to heading in rice. *G3: Genes, Genomes, Genetics.* **9**(5): 1655-1662.
- Kovačević, A., Pavlov, J., Stevanović, M., Grčić, N., Mladenović, M., Deli, N., Knežević, N. (2022). Effect of reciprocal crosses on grain yield and other agronomic traits in maize. *Genetika.* **54**(3): 1365-1374.
- Lehermeier, C., Teyssedre, S., Schon, C.C. (2017). Genetic gain increases by applying the usefulness criterion with improved variance prediction in selection of crosses. *Genetics.* **207**(4): 1651-1661.
- Levene, H. (1960). Robust Tests for Equality of Variances. In: [Olkin, I. et al. (Eds.)], Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling, Stanford University Press, Stanford, USA, pp. 278-292.
- Mackay, I.J., Cockram, J.C., Howell, P., Powell, W. (2020). Understanding the classics: The unifying concepts of transgressive segregation, inbreeding depression and heterosis and their central relevance for crop breeding. *Plant Biotechnol J.* **19**: 26-34.
- Mann, C.E. and Pollmer, W.G. (1981). Reciprocal-cross differences between maize hybrids of inbred lines from different gene pools. *Maydica.* **26**(4): 263-271.
- Melchinger, A.E., Geiger, H.H., Schnell, F.W. (1985). Reciprocal differences in single-cross hybrids and their F2 and backcross progenies in maize. *Maydica.* **30**: 395-405.

- Ongom, P.O., Fatokun, C., Togola, A., Oyeboode, O.G., Ahmad, M.S., Jockson, I.D., Bala, G., Boukar, O. (2021). Genetic worth of multiple sets of cowpea breeding lines destined for advanced yield testing. *Euphytica*. **217**: 30. <https://doi.org/10.1007/s10681-020-02763-y>.
- Pollmer, W.G., Klein, D., Dhillon, B.S. (1979). Differences in reciprocal crosses of maize inbred lines diverse for protein content. *Euphytica*. **28(2)**: 325-328.
- R Core Team. (2024). R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <https://www.R-project.org>.
- Reddy, B.R., Lal, H., Pandey, M., Raj, N. (2025). Stability and diversity of elite lines of vegetable cowpea (*Vigna unguiculata* ssp. *unguiculata* L.). *Legume Research*. **48(8)**: 1281-1286. doi: 10.18805/LR-5140.
- Tripathi, K., Gore, P.G., Ahlawat, S.P., Tyagi, V., Semwal, D.P., Gautam, N.K., Rana, J.C., Kumar, A. (2019). Cowpea genetic resources and its utilization: Indian perspective-A review. *Legume Research*. **42(4)**: 437-446. doi: 10.18805/LR-4146.
- Vaggar, S., Kumar, U., Prasad, K., Yadav, L.M., Pramila, Sinha, B.M. (2022). Study of genetic divergence for yield and quality traits in cowpea [*Vigna unguiculata* (L.) Walp.]. *Legume Research*. **45(4)**: 410-414. doi:10.18805/LR-4297.