Electronic Journal of Plant Breeding, 9(4): 1295-1302 (Dec 2018) DOI: 10.5958/0975-928X.2018.00162.X
ISSN 0975-928X

Research Article

Genetic variability for seedling characters in lentil under salinity stress

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(Received: 27 Jun 2018; Revised: 23 Nov 2018; Accepted: 11 Dec 2018)

Abstract
A laboratory experiment was conducted to study genetic variation in 10 genotypes of lentil (*Lens culinaris* M.) under four levels of salinity (0, 20, 40 and 60 mM NaCl). 15 seeds of each genotype were placed in sterilized petridishes layered with autoclaved germination papers at 24±2^{0}C in the culture room. The ANOVA over different salinity levels revealed significant variation among genotypes, salinity levels and their interactions for all the characters except germination percentage. The reductions in mean values were severe in germination percentage, plumule length, radicle fresh weight, plumule dry weight, radicle dry weight, and seedling vigour index under high salinity. The highest GCV, PCV and genetic advance was observed in plumule to radicle length ratio followed by radicle length and heritability in broad sense was generally high for plumule fresh weight, radicle length and seedling length across the salinity levels, indicating that variation was highest for these characters among the genotypes.

Key words
Lentil, Salinity, Variation, GCV, Seedling.

Introduction

Lentil (*Lens culinaris* M.) is one of the most important grain legume and nitrogen fixing crop. It is mainly cultivated in semi-arid regions of the world particularly in the Indian sub-continent and the dry areas of Middle East (Malik, 2005). Salinity is one of the most serious factors that hamper the productivity of agricultural crops, with adverse effects on germination, plant vigour and crop yield (Munns and Tester, 2008) particularly in arid and semi-arid regions of the world (Ahmed, 2009). Salt affected soils are distributed throughout the world and no continent is free from the problem (Brady and Weil, 2002). Soil salinity designates a condition in which the soluble salt content of the soil reaches a level harmful to crops through the reduced osmotic potential of the soil solution and the toxicity of specific ions. These soluble salts may be from those present in the original soil profile or transported to the profile by irrigation water containing an unusual high concentration (Ates and Tekeli, 2007). All these factors manifest themselves by morphological, physiological and metabolic modifications in plant such as decrease in seed germination, shoot and root length, alterations in the integrity of cell membranes, changes in different enzymatic activities and photosynthesis. Seed germination is a complicated process and is sensitive to salt stress (Begum *et al.*, 2013). Salinity of the environment influence seed germination by reducing the osmotic potential and toxicity of specific ions such as sodium and chlorine, as well as reducing essential nutrients such as calcium and potassium. Salinity reduces the ability of plants to take up water, leading to metabolic effect that reduces plant growth. The deleterious consequences of high salt concentrations in the external solution of plant cells are hyper-osmotic shock and ionic imbalance. Although, salt stress affects all growth stages of a plant but seed germination and seedling growth stages are known to be more sensitive for most of the plant species. Germination failures on saline soils are often the results of high salt concentrations in the seed planting zone. A study on variability available in the genotypes is the pre-requisite for initiating a varietal development programme. Hence, analyzing the nature and magnitude of the heritable genetic variation present in the genotypes is necessary. The crosses between parents with maximum genetic divergence are generally the most responsive for genetic improvement (Arunachalam, 1981). Genetic diversity can be evaluated with morphological traits, seed protein, isozymes and DNA markers. Conventionally, it is estimated by analysis of variance using morphological traits. Evaluation of lentil genotypes for resistance to salt stress is very important. Therefore, the general objective of this study was to assess the genetic variability for salinity tolerance among some lentil genotypes.

Materials and Methods

The laboratory experiment was carried out at Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner (Sri Karan Narendra Agriculture University, Jobner-303329, Rajasthan) in December, 2016 at a temperature 24±2 ^{0}C. The seeds of ten genotypes of lentil namely, RLG–5, RLG–195, RLG–234, RLG–250, RLG–254, RLG–256, RLG–258, SAPNA, DPL–58 and L–4076 were used for evaluation. Prior to germination, the sorted uniform seeds were surface sterilized with 0.1% mercuric chloride for 1 minute and washed 3 times under running tap water followed by washing with double distilled water. Four salinity levels viz., 0.0, 20, 40 and 60 mM NaCl were prepared by dissolving 0, 292.2, 584.4 and 876.6 mg of NaCl salt in 250 ml of double distilled water, respectively to be used in experiment and designated as S_0, S_1, S_2 and S_3, respectively. The experiment with ten genotypes and four NaCl concentrations was laid out in randomized block design (RBD) with three replications. Fifteen seeds of each genotype were germinated in sterilized (165^{0}C for 4 hours in hot air oven) petridishes of 9 cm diameter layered with autoclaved (15 psi and 121^{0}C for 20 minutes) germination papers and then moistened with 3 ml of test solutions daily after removing previous day solution. The set was maintained in dark for first two days. Five seedlings were randomly selected from each petridish to record the observations on various seedling characters. The methods used for recording observations are described below:

A seed was considered as germinated at the emergence of both radicle and plumule up to 2 mm length (Chartzoulakis and Klapaki, 2000). The germination was recorded on 8th day of planting and germination percentage was determined by using the following formula (Aniat *et al.*, 2012):

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

On 8th day the plumule, radicle and seedling length of germinated seeds was recorded. For this, five seedlings were randomly selected and divided into two parts viz., plumule and radicle and length was measured using measuring scale in centimeter and averaged. The hypocotyl length was included in plumule length. The seedling length was recorded by adding plumule length and radicle length of the same five seedlings which were selected already from each replication and in each treatment.

The plumule to radicle length ratio of seedling was obtained by dividing plumule length with radicle length (Kagan *et al.*, 2010).

The fresh weight of plumules and radicles of the same five seedlings was weighed in milligram by using a sensitive electronic balance and averaged.

The plumules and radicles which were taken for fresh weight were kept into paper bags with proper labelling and kept in oven at 65^{0}C for 48 hours for drying. After drying, the dried plumules and radicles were weighed by sensitive electronic balance in milligram and average was recorded.

The seedling vigour index was determined by multiplying the sum total of mean length of plumule and radicle of a seedling with concerned germination percentage by using the following formula (Iqbal and Rahmati, 1992):

Seedling Vigour Index = (Plumule length + Radicle length) x (Germination %)

The data obtained were subjected to analysis of variance following standard statistical methods (Panse and Sukhatme, 1985) and significant differences among the mean values were compared by least significant difference (LSD) test ($P<0.05$). Variability parameters were calculated using various formulae given by Burton (1952) and Johnson *et al.* (1955).

Results and Discussion

The analysis of variance over different salinity levels (Table 1) indicated that the genotypes, salinity levels and genotype × salinity interaction exhibited significant mean sum of squares for all the characters except germination percentage due to genotypes. This indicated differential response of genotypes to salinity levels for all the characters and presence of inherent variability among the genotypes. Analysis of variance for individual salinity level indicated significant differences among the genotypes in all the levels for most of the characters.

Variability parameters and other attributes of different characters are presented in Table 2.

The germination percentage ranged from 91.11 % to 100 % in S_0, 88.89% to 97.78% in S_1, 84.44% to 95.55% in S_2 and 75.55 % to 86.67 % in S_3, indicating that salinity adversely affected seed germination. The GCV was 0.73 %, 2.22 %, 3.24 % and 3.83 % in S_0, S_1, S_2 and S_3 level, respectively. The PCV was 4.11 %, 5.10 %, 5.63 % and 6.46 % in S_0, S_1, S_2 and S_3 level, respectively. Heritability was 3.15 %, 18.99 %, 33.12 %, and 35.15 % in S_0, S_1, S_2 and S_3, respectively. It was lowest in S_0, and highest in S_3. Genetic advance as percentage of mean was 0.27 %, 2.00 %, 3.84 % and 4.68 % in S_0, S_1, S_2 and S_3, respectively.

It ranged from 6.55 cm to 8.09 cm in S_0, 5.51 cm to 6.51 cm in S_1, 4.57 cm to 6.61 cm in S_2 and 4.50 cm to 6.21 cm in S_3. The GCV was 5.51 %, 5.17 %, 9.51 % and 8.00 % in S_0, S_1, S_2 and S_3, respectively and PCV was 8.71 %, 8.88 %, 11.65 % and 9.84 % in S_0, S_1, S_2 and S_3, respectively. Heritability was 39.97 %, 33.85 %, 66.64 %, and 66.17 % in S_0, S_1, S_2 and S_3, respectively. The genetic advance as percentage of mean was 7.17 %, 6.19 %, 15.99 % and 13.41 % in S_0, S_1, S_2 and S_3, respectively.

The radicle length ranged from 5.79 cm to 9.43 cm in S_0, 3.61 cm to 5.33 cm in S_1, 3.88 cm to 6.39 cm in S_2 and 4.33 cm to 7.97 cm in S_3 level. It decreased from S_0 (7.81 cm) to S_1 (4.45 cm) and further increased in S_2 (4.66 cm) and in S_3 (6.14 cm). The GCV was 14.70 %, 10.78 %, 13.45 % and 16.14 % in S_0, S_1, S_2 and S_3, respectively. The PCV was 16.93 %, 16.10 %, 18.67 % and 19.43 % in S_0, S_1, S_2 and S_3, respectively. Heritability was 75.37 %, 44.86 %, 51.91 %, and 68.96 % in S_0, S_1, S_2 and S_3, respectively. Regarding genetic advance as percentage of mean, an irregular trend was observed with increase in salinity level. It was 26.28 %, 14.88 %, 19.96 % and 27.61 % in S_0, S_1, S_2 and S_3, respectively.

The seedling length ranged from 12.89 cm to 17.00 cm in S_0, 9.21 cm to 11.83 cm in S_1, 9.34 cm to 11.82 cm in S_2 and 10.41 cm to 13.95 cm in S_3. The range was wider in S_0 and narrow in S_2. The GCV was 8.24 %, 7.00 %, 6.77 % and 8.82 % in S_0, S_1, S_2 and S_3, respectively and PCV was 10.34 %, 9.83 %, 9.69 % and 10.46 % in S_0, S_1, S_2 and S_3, respectively. Heritability was 63.44 %, 50.78 %, 48.80 %, and 71.12 % in S_0, S_1, S_2 and S_3, respectively. Genetic advance was 13.52 %, 10.28 %, 9.75 % and 15.32 % in S_0, S_1, S_2 and S_3 level, respectively. It was lowest in S_2 and highest in S_3.

The plumule to radicle length ratio ranged from 0.73 to 1.23 in S_0, 1.19 to 1.66 in S_1, 0.85 to 1.71 in S_2 and 0.71 to 1.45 in S_3. The GCV was 15.29 %, 8.78 %, 16.66 % and 22.76 % in S_0, S_1, S_2 and S_3, respectively and PCV was 18.07 %, 15.34 %, 22.69 % and 27.02 % in S_0, S_1, S_2 and S_3, respectively. Heritability was 71.62 %, 32.75 %, 53.92 %, and 70.93 % in S_0, S_1, S_2 and S_3, respectively. Genetic advance was 26.66 %, 10.35 %, 25.21 % and 39.48 % in S_0, S_1, S_2 and S_3, respectively.

It ranged from 51.07 mg to 69.52 mg in S_0, 42.32 mg to 60.60 mg in S_1, 38.81 mg to 52.20 mg in S_2 and 37.91 mg to 54.50 mg in S_3 level. The range was wider in S_0 and shorter in S_2. The GCV was 8.47 %, 11.00 %, 9.73 % and 11.76 % in S_0, S_1, S_2 and S_3, respectively. The PCV was 12.55 %, 12.74 %, 11.02 % and 13.30 % in S_0, S_1, S_2 and S_3, respectively. Heritability was 45.56 %, 74.59 %, 77.99 %, and 78.17 % in S_0, S_1, S_2 and S_3, respectively. Genetic advance as percentage of mean was 11.77 %, 19.57 %, 17.70 % and 21.42 % in S_0, S_1, S_2 and S_3 level, respectively. It was lowest in S_0 and highest in S_3.

It ranged from 38.14 mg to 62.37 mg in S_0, 29.23 mg to 38.13 mg in S_1, 27.81 mg to 39.85 mg in S_2 and 20.97 mg to 39.40 mg in S_3. The range was wider in S_0 and shorter in S_1. The GCV was 14.55 %, 8.64 %, 9.56 % and 16.22 % in S_0, S_1, S_2 and S_3, respectively. The PCV was 16.06 %, 14.24 %, 15.26 % and 19.13 % in S_0, S_1, S_2 and S_3, respectively. Heritability was 82.01 %, 36.81 %, 39.28 %, and 71.81 % in S_0, S_1, S_2 and S_3, respectively. Genetic advance as percentage of mean was 27.14 %, 10.80 %, 12.35 % and 28.31 % in S_0, S_1, S_2 and S_3 level, respectively. It was lowest in S_1 and highest in S_3.

Results of plumule dry weight revealed that it ranged from 5.37 mg to 7.55 mg in S_0, 4.56 mg to 6.11 mg in S_1, 4.32 mg to 5.87 mg in S_2 and 4.19 mg to 5.32 mg in S_3 salinity level. The range was wider in S_0 and shorter in S_3 level. The GCV was 9.27 %, 7.04 %, 8.75 % and 5.75 % in S_0, S_1, S_2 and S_3 level, respectively. The PCV was 15.01 %, 11.64 %, 12.19 % and 9.98 % in S_0, S_1, S_2 and S_3 level, respectively. Heritability was 38.18 %, 36.55 %, 51.52 %, and 33.18 % in S_0, S_1, S_2 and S_3 level, respectively. Genetic advance was 11.81 %, 8.76 %, 12.94 % and 6.82 % in S_0, S_1, S_2 and S_3 level, respectively. It was lowest in S_3 and highest in S_2.

The radicle dry weight ranged from 2.37 mg to 3.64 mg in S_0, 2.33 mg to 2.81 mg in S_1, 1.80 mg to 2.75 mg in S_2 and 1.76 mg to 3.07 mg in S_3 salinity level. The range was wider in S_3 and shorter in S_1 salinity level. The GCV was 12.24 %, 7.05 %, 10.93 % and 14.23 % in S_0, S_1, S_2 and S_3 level, respectively. The PCV was 13.91 %, 9.21 %, 15.34 % and 18.26 % in S_0, S_1, S_2 and S_3 level, respectively. Heritability was 31.16 %, 53.94 %, 41.95 %, and 75.24 % in S_0, S_1, S_2 and S_3 level, respectively. Genetic advance was 6.67 %, 11.79 %, 10.21 % and 20.24 % in S_0, S_1, S_2 and S_3 level, respectively. It was lowest in S_0 and highest in S_3.

The seedling vigour index ranged from 1260.52 to 1567.65 in S_0, 835.99 to 1078.65 in S_1, 791.04 to 1077.23 in S_2 and 810.66 to 1176.56 in S_3. The GCV was 5.80 %, 7.79 %, 7.65 % and 11.33 % in S_0, S_1, S_2 and S_3 level, respectively. The PCV was 10.39 %, 10.61 %, 11.81 % and 13.06 % in S_0, S_1, S_2 and S_3 level, respectively. Heritability was 31.16 %, 53.94 %, 41.95 %, and 75.24 % in S_0, S_1, S_2 and S_3 level, respectively. Genetic advance was 6.67 %,

11.79 %, 10.21 % and 20.24 % in S_0, S_1, S_2 and S_3 level, respectively.

Development of salinity tolerant lines is an ideal way to mitigate the adverse effects of salinity on lentil cultivation. Studies on variability and screening for salinity tolerance in lentil are limited as compared to other legume crops especially for seedling traits. The present study is therefore, an attempt in this direction. The findings emanating from the present investigation are discussed here in the light of available literature.

Genotype × environment interaction is a common phenomenon present in crop plant species (Allard and Bradshaw, 1964). In the present investigation the analysis of variance over different salinity levels showed that the mean squares due to genotypes and salinity levels were significant, indicating significant differences among genotypes and the effect of salinity on the genotypes except for germination percentage in which case the genotypes did not differ significantly. The genotype x salinity interaction was found significant for all the characters studied which indicated differential response of genotypes to salinity. Similar results have also been reported in lentil by Azene *et al.* (2014). Since the genotype x salinity interaction was significant, it would be worthwhile to compare the genotypes over different salinity levels for different characters rather than the pooled mean alone. In view of this, the analysis of variance was carried out for each salinity level separately. Analysis of variance in the individual salinity level revealed significant differences among the genotypes for most of the characters under study. Similar findings were also reported in lentil by Tesfaye *et al.* (2015). This indicated presence of sufficient genetic variability in the genotypes used.

It might be concluded that variation due to salinity on different characters were not uniform as some characters were influenced more while others less. This reduction in mean performance was due to salts of different nature and concentration because increased water potential, restricted the movement of water towards the seed surface (Houimli *et al.*, 2008). Variation for germination and seedling characteristics at different salinity levels was widely reported in mung bean (Swarnakar, 2016), in lentil, chickpea and faba bean (Arslan *et al.*, 2016), in cowpea (Haleem, 2015), in *Pisum sativum* var. abyssinicum and *Lathyrus sativus* (Tsegay and Gebreslassie, 2014), in moth bean and mung bean (Saroj and Soumana, 2014) and in fenugreek (Jat *et al.*, 2014). The salinity gradient adversely affected the mean values of almost all the characters, except radicle length. The mean values of radicle length were higher at 0 mM and 60 mM salinity than at 20 mM and 40 mM salinity. Such stimulatory effect of salinity has been reported earlier in lentil (Sariye and Ercan, 2015). It might be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings and lower water availability (Xiong and Zhu, 2002). In general, the PCV values were higher than GCV values for all the characters indicating role of environment on the character expression. The differences were however, low for all the traits. Further, highest GCV and PCV values were observed in plumule to radicle length ratio followed by radicle length, indicating that variation among the genotypes was highest for these traits. The PCV and GCV values were lowest in germination percentage followed by the plumule length, indicating these to be the lowest variable characters. Comparison for each character across the salinity levels indicated that mean values decreased with increasing salinity levels the GCV and PCV values increased, this may indicate a complex response mechanism which brought variations among genotypes and possibility of selection of responsive genotypes. This is particularly true for germination percentage. No specific trend was observed for plumule length, seedling length, plumule fresh weight, plumule dry weight and seedling vigour index i.e. the GCV for plumule dry weight is higher in S_0 than decreased in S_1 than again increased in S_3 as compared with S_2. The increase in GCV and PCV was more in germination percentage and radicle dry weight as reported earlier by Jat (2009). Heritability in broad sense was generally high for plumule fresh weight, radicle length and seedling length across the salinity levels. This is in agreement with the reports of Gupta (1994) and Jat *et al.* (2014). Genetic advance was highest for plumule to radicle length ratio followed by radicle length and was lowest for germination percentage. Similar to GCV and PCV, the GA also showed linear increase with salinity gradient in germination percentage. While, no specific trend was observed in the rest of characters studied. Increase in the magnitude of genetic advance across the salinity gradient indicates increase in the inherent variation in the response of genotype to salinity and a possibility of selection of suitable genotypes at higher salinity. Similar observations were also noted earlier by Gupta (1994).

Highest GCV and PCV values were observed in plumule to radicle length ratio followed by radicle length and heritability in broad sense values was generally high for plumule fresh weight, radicle length and seedling length across the salinity levels and genetic advance as percentage of mean was highest for plumule to radicle length ratio followed by radicle length. Hence, it is suggested that major emphasis should be given on these characters

having wide genetic parameters during selection. It would be ideal if variability studies for germination and seedling characters to be done at some higher concentrations of salts to identify genotypes for salt tolerance for further breeding programmes.

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Electronic Journal of Plant Breeding, 9(4): 1295-1302 (Dec 2018) DOI: 10.5958/0975-928X.2018.00162.X
ISSN 0975-928X

Table 1. The ANOVA over different salinity levels for various traits (MSS)

Characters	Source of variation with degree of freedom				
	Genotypes (df=9)	Salinity levels (df=3)	Replication/ Salinity (df=8)	Genotype × Salinity (df=27)	Error (df=72)
Germination percentage	28.853	1358.087**	28.489	35.767**	16.997
Plumule length (cm)	1.64**	21.163**	0.216	0.437**	0.172
Radicle length (cm)	3.767**	72.767**	0.501	2.173**	0.38
Seedling length (cm)	3.69**	149.6**	0.305	3.213**	0.594
Plumule to radicle length ratio	0.244**	1.56**	0.045	0.078**	0.024
Plumule fresh weight (mg)	212.933**	1726.973**	10.974	44.461**	13.155
Radicle fresh weight (mg)	168.908**	1864.03**	11.1	49.739**	12.195
Plumule dry weight (mg)	1.583**	12.293**	0.339	0.507*	0.26
Radicle dry weight (mg)	0.892**	2.71**	0.016	0.111**	0.049
Seedling vigour index	28113.666**	1970677.79**	6608.324	30478.141**	7888.484

* and ** represent significant at 5% and 1% level of significance, respectively

Electronic Journal of Plant Breeding, 9(4): 1295-1302 (Dec 2018) DOI: 10.5958/0975-928X.2018.00162.X
ISSN 0975-928X

Table 2. The general mean, range, genotypic and phenotypic coefficient of variation (GCV & PCV), heritability (in broad sense) and genetic advance (GA) over different salinity levels for different characters

Salinity Levels	Germination percentage						Plumule length (cm)					
	Mean	Range	GCV	PCV	h^2 (%)	GA (%)	Mean	Range	GCV	PCV	h^2 (%)	GA (as % of mean)
S_0	96.22	91.11 - 100	0.73	4.11	3.15	0.27	7.37	6.55 - 8.09	5.51	8.71	39.97	7.17
S_1	93.11	88.89 - 97.78	2.22	5.1	18.99	2.00	6.06	5.51 - 6.51	5.17	8.88	33.85	6.19
S_2	89.33	84.44 - 95.55	3.24	5.63	33.12	3.84	5.77	4.57 - 6.61	9.51	11.65	66.64	15.99
S_3	80.67	75.55 - 86.67	3.83	6.46	35.15	4.68	5.46	4.50 - 6.21	8.00	9.84	66.17	13.41
	Radicle length (cm)						**Seedling length (cm)**					
S_0	7.81	5.79 - 9.43	14.7	16.93	75.37	26.28	15.19	12.89 - 17.00	8.24	10.34	63.44	13.52
S_1	4.45	3.61 - 5.33	10.78	16.10	44.86	14.88	10.51	9.21 - 11.83	7.00	9.83	50.78	10.28
S_2	4.66	3.88 - 6.39	13.45	18.67	51.91	19.96	10.43	9.34 - 11.82	6.77	9.69	48.8	9.75
S_3	6.14	4.33 - 7.97	16.14	19.43	68.96	27.61	11.6	10.41 - 13.95	8.82	10.46	71.12	15.32
	Plumule to radicle length ratio						**Plumule fresh weight (mg)**					
S_0	0.97	0.73 - 1.23	15.29	18.07	71.62	26.66	59.9	51.07 - 69.52	8.47	12.55	45.56	11.77
S_1	1.39	1.19 - 1.66	8.78	15.34	32.75	10.35	47.29	42.32 - 60.60	11.00	12.74	74.59	19.57
S_2	1.28	0.85 - 1.71	16.66	22.69	53.92	25.21	43.67	38.81 - 52.20	9.73	11.02	77.99	17.7
S_3	0.93	0.71 - 1.45	22.76	27.02	70.93	39.48	44.23	37.91 - 54.50	11.76	13.3	78.17	21.42
	Radicle fresh weight (mg)						**Plumule dry weight (mg)**					
S_0	47.48	38.14 - 62.37	14.55	16.06	82.01	27.14	6.06	5.37 - 7.55	9.27	15.01	38.18	11.81
S_1	33.70	29.23 - 38.13	8.64	14.24	36.81	10.8	5.09	4.56 - 6.11	7.04	11.64	36.55	8.76
S_2	31.66	27.81 - 39.85	9.56	15.26	39.28	12.35	4.74	4.32 - 5.87	8.75	12.19	51.52	12.94
S_3	30.48	20.97 - 39.40	16.22	19.13	71.81	28.31	4.68	4.19 - 5.32	5.75	9.98	33.18	6.82
	Radicle dry weight (mg)						**Seedling vigour index**					
S_0	2.98	2.37 - 3.64	12.24	13.91	77.43	22.19	1459.9	1260.52 - 1567.65	5.80	10.39	31.16	6.67
S_1	2.55	2.33 - 2.81	7.05	9.21	58.47	11.10	978.27	835.99 - 1078.65	7.79	10.61	53.94	11.79
S_2	2.35	1.80 - 2.75	10.93	15.34	50.71	16.03	932.19	791.04 - 1077.23	7.65	11.81	41.95	10.21
S_3	2.34	1.76 - 3.07	14.23	18.26	60.76	22.85	936.62	810.66 - 1176.56	11.33	13.06	75.24	20.24