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Comparative study of physiological compound and variability studies in different *Allium* species

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Abstract

Onion (*Allium cepa* L.), a member of Alliaceae family, is one of the important export oriented vegetable crop cultivated across the world. In India, it is the most popular and consumed spices in the daily human diet. In world, India ranks first in area and second in onion production (Kumar *et al.*, 2015). Most cultivated *Alliums* lack many important traits, including resistance to pests and environmental stresses. Both producers and consumers face severe problems during crop failure due to price fluctuations. Underutilized *Allium* genotypes are an essential source of various biochemical components, vitamins, micronutrients and, thus, these are valuable component to attain nutritional security. Underutilized *Alliums* spp. can be used as a supplement to onion and garlic during crop failures. Current efforts and employment of both classical and novel tools for genetic and physiological studies are expected to accelerate improvements in terms of distribution, yield, and quality of these important crops. The present investigation was framed and conducted with 40 *Allium* genotypes laid out in RBD in three replications at ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, Pune, Maharashtra, during rabi season of 2017-18. Physiological traits namely, chlorophyll, leaf membrane stability index, Relative water content, TSS, Leaf area, Dry matter content and Moisture content were estimated from leaf sample. *Allium tuberosum* showed the highest total chlorophyll content (3.92g/ml), *Allium angulosum* showed the highest membrane stability index (34.61%) *Allium angulosum* showed the highest relative water content (84.04%) *Allium macranthum* showed the highest TSS (11.33%) *Allium cepa* showed the highest leaf area (19.53 cm²) *Allium chinense* showed the highest dry matter content (40.78%), *Allium angulosum* showed the highest moisture content (86.13%) physiological content in underutilized *Alliums* was significantly higher compared to cultivated *Alliums* which provide ample scope for selection of promising genotypes under study.

Keywords: Cucumber, boron, yield, quality, konkan

Introduction

Allium is a large genus which is widely distributed in the Central Asia, North America and Indian Himalayan regions (Stearn, 1992; Negi and Pant, 1992) [7, 4]. Several *Allium* species are grown by local tribes in wild or semi-domesticated forms in India. These species are utilized as vegetables, condiments and medicinal plants to fulfil their daily needs (Negi, 2006; Pandey *et al.*, 2008) [4]. It includes more than 700 species widely distributed all over the world. *Allium* species may differ in form and taste, but they are close in biochemical and physiological contents. They constitute important ingredients in many European and Asian diets and they have been known for their medicinal properties. In order to optimize better utilization of *Allium* germplasm this work was planned with objective of characterizing *Allium* germplasm based on morphological physiological and biochemical characters to identify major components of variation and grouping of various *Allium* species based on them. The goal was to identify the differences among the accessions and major variables which leads to the identification of possible groups and relationships among accessions.

Material and Method

The study was conducted with thirty seven genotype of four species of underutilised *Alliums* viz. *Allium angulosum*, *Allium chinensis*, *Allium macranthum*, *Allium tuberosum* were collected from Austria, Netherland and different part of India. Three onion varieties viz. Bhima Kiran, Bhima Shakti and Bhima Shweta from DOGR Pune Maharashtra laid out in

RBD in three replication at ICAR-Directorate of Onion and Garlic Research, Rajgurunagar, Pune, Maharashtra, during rabi season of 2017-18. A total of 40 genotypes of each *Allium* accession were cultured in three replicates, each in a plot of 1.0 × 2.0 m with distance of 25 × 20 cm for each plant. Physiological parameters were analysed at DOGR Pune and NRC Grapes, Pune. The analysis of variance was carried out for each character as per method of Panse and Sukhatme (1967)^[5].

Physiological parameters

Estimation of chlorophyll content

Chlorophyll content was estimated as per the method described by Hiscox and Israelstam (1979). The procedure for estimation of chlorophyll content in plants is based on the absorption of light by chlorophyll extracts prepared by incubating the leaf tissues in DMSO (dimethyl sulfoxide). The absorbance of the known volume of solution containing known quantity of leaf tissue at two respective wavelengths (663 and 645 nm) was determined for chlorophyll content. Total chlorophyll content was estimated using the formula given by Arnon (1949). Fifty mg fresh leaf samples were added to the test tubes containing 4.0 ml DMSO. Tubes were kept in dark for 4 h at 65 °C. Then the samples were taken out cooled at room temperature and the absorbance was recorded at 663 and 645 nm using DMSO as blank. The values thus obtained were in µg/ ml of extract (solvent). Values in mg/ g fresh weight were obtained by multiplying the above values with V/ W × 1000, where V is volume of extract and W is dry weight of sample.

$$\text{Total chlorophyll content (mg gDW}^{-1}\text{)} = [20.2 \times A_{645} + 8.02 \times A_{663}] \times (V/W) \times 1000$$

Where,

A₆₄₅ = Absorbance values at 645 nm

A₆₆₃ = Absorbance values at 663 nm

Estimation of relative water content (%)

Relative water content (RWC) was measured according to the method of Tambussi *et al.* (2005). The fully expanded top most leaf were collected from the plant following the standard procedure and immediately shifted to an ice bucket and transported to laboratory to avoid moisture loss. The samples were weighed quickly to record the fresh weight. The samples were hydrated to full turgidity by floating on de-ionized water in a closed petridish for 6 hours at the room temperature. After 6 hours leaf was removed from water, its surface blotted off to remove surface water. Leaf was reweighted to obtain turgid weight. The samples were dried in a hot air oven at 80°C for 25 hours. The dry weight of the samples weighted after proper drying (till the weight become constant). Relative water content (%) was computed using the following equation-

$$\text{RWC (\%)} = [(\text{Fresh weight} - \text{Dry weight}) / (\text{Turgid weight} - \text{Dry weight})] \times 100$$

Estimation of leaf membrane stability index (%)

Membrane stability index (MSI) was measured according to the method of Sairam *et al.* (1994). Leaf sample 0.5gm of uniform size was taken in test tubes containing 10ml of double distilled water in two sets. Test tubes in one set were kept at 40°C in a water bath for 30 minutes and electrical conductivity of water containing sample was measured (C1)

using a conductivity bridge. Test tubes in other sets were incubated at 100°C in the boiling water bath for 15 minutes, and their electrical conductivity was measured as above (C2). Membrane stability index (%) was calculated using the following equation-

$$\text{MSI (\%)} = [1 - C1/C2] \times 100$$

Where,

C1- Electrical conductivity of sample at 40°C

C2- Electrical conductivity of sample at 100°C

Dry matter content (%)

The leaves sample of individual genotype drawn from each replication the sample was kept in the paper bag having holes on both the sides. The bags were kept in hot air oven at 60°C until reaching the constant weight. Dry weight of leaves was divided by fresh weight of leaves and then multiplied by hundred to obtain the dry matter content for each sample.

$$\text{DM (\%)} = \frac{\text{Dry weight of plant}}{\text{Fresh weight of plant}} \times 100$$

TSS (%)

The total soluble solids content of *Allium* leaf extract was directly measured by the Zeiss Hand Refractometer (0-30) and value obtained was corrected at 2000C. (A.O.A.C, 1984)

Leaf area (cm²)

Leaf area was measured by leaf area meter. And expressed in centimeter square.

Result and Discussion

Total chlorophyll (g/ml)

The total chlorophyll content of genotypes and species as well under study is presented in Table 1 and 2. Total chlorophyll content varied from 1.27g/ml to 7.69. *Allium tuberosum* NG-3183 has significantly highest total chlorophyll (7.69g/ml) The lowest total chlorophyll content (1.27g/ml) was recorded in *Allium macranthum* NMK-3237 Among the species *Allium tuberosum* showed the highest total chlorophyll content (3.92g/ml) followed by *Allium cepa* (3.78g/ml) and *Allium chinense* (2.05g/ml). The lowest total chlorophyll content (1.29g/ml) was recorded in *Allium angulosum*. This finding is in close proximity with the results of Stajner *et al.* (2003).

Chlorophyll 'a' (g/ml)

The chlorophyll 'a' content of different genotypes and species under study is presented in Table 1 and 2. Chlorophyll 'a' content varied from 0.96 g/ml to 5.87. *Allium tuberosum* NG-3183 has significantly highest chlorophyll 'a' content (5.87 g/ml) The lowest chlorophyll 'a' content (0.96 g/ml) was recorded in *Allium macranthum* NMK-3237 Among the species *Allium tuberosum* showed the highest chlorophyll 'a' content (3.03g/ml) followed by *Allium cepa* (2.89 g/ml). The lowest chlorophyll 'a' content (0.99 g/ml) was recorded in *Allium angulosum* (Table 2).

Chlorophyll 'b' (g/ml)

The chlorophyll 'b' content of different genotypes and species under study is presented in Table 1 and 2. Chlorophyll 'b' content varied from 0.27 g/ml to 1.82. *Allium tuberosum* NG-3183 has significantly highest chlorophyll 'b' content (1.82 g/ml). The lowest chlorophyll 'b' content (0.27 g/ml) was recorded in *Allium macranthum* NMK-3236. Among the

species *Allium cepa* showed the highest chlorophyll 'b' content (0.90 g/ml) followed by *Allium tuberosum* (0.87 g/ml). The lowest chlorophyll 'b' content (0.3 g/ml) was recorded in *Allium angulosum* (Table 2).

Relative water content (%)

The relative water content of different genotypes and species as well under study is presented in 1 and 2. It reveals that relative water content varied from 55.90% to 90.64%. *Allium tuberosum* Rottl Ex-spr kuchaai CGN-16412(f) has significantly highest relative water content (90.64%). The lowest relative water content (55.90%) was recorded in Bhima Shweta. Among the species *Allium angulosum* showed the highest relative water content (84.04%) followed by *Allium tuberosum* (83.61%) and *Allium macranthum* (71.03%). The lowest relative water content (58.72%) was recorded in *Allium cepa* (Table 2) Relative water content in underutilized *Alliums* was significantly higher compared to *Allium cepa* (Bhima Shakti, Bhima Kiran). This finding is in close proximity with the results of Khalid *et al.* (2014) [12].

Membrane stability index (%)

The membrane stability index of different genotypes and species under study is presented in Table 1 and 2. Membrane stability index varied from 0.23% to 58.08%. *Allium tuberosum* EC-60748 has significantly highest membrane stability index (58.08%). The lowest membrane stability index (0.23%) was recorded in *Allium macranthum* NMK-3236. Among the species *Allium angulosum* showed the highest membrane stability index (34.61%) followed by *Allium tuberosum* (22.5%) and *Allium chinense* (7.47%). The lowest membrane stability index (7.21%) was recorded in *Allium macranthum* (Table 2).

Dry matter content (%)

The dry matter percentage of genotypes and species as well under study is presented in Table 1 and 2. It reveals that *Allium macranthum* NMK-3233 has significantly highest dry matter content (50.00%) The lowest dry matter content (12.87%) was recorded in *Allium tuerosum* NMK-3219. Among the species *Allium chinense* showed the highest dry

matter content (40.78%) followed by *Allium macaranthum* (39.9%). The lowest dry matter content (13.87%) was recorded in *Allium angulosum* (Table 2). This finding is in close proximity with the results of Adamczewka *et al.* (2010) [1].

Moisture content (%)

Moisture content varied from 50.00% to 87.13%. Moisture content in different genotypes and species is presented in Table 1 and 2. *Allium tuerosum* NMK-3219 has significantly highest dry matter content (87.13%). The lowest moisture content (50.00%) was recorded in *Allium macranthum* NMK-3233. Among the species *Allium angulosum* showed the highest moisture content (86.13%) followed by *Allium cepa* (84.51%) and *Allium tuberosum* (84.39%). The lowest moisture content (60.1%) was recorded in *Allium macranthum*. (Table 2).

Total soluble solid (%)

The total soluble solid of different genotypes and species under study is presented in Table 1 and 2. Total soluble solid varied from 5.00(%) to 14.27(%). *Allium macranthum* NMK-3216 has significantly highest total soluble solid (14.27%) The lowest total soluble solid (5.00%) was recorded in *Allium tuberosum* kazakhstan All-1587. Among the species *Allium macranthum* showed the highest TSS (11.33%) followed by *Allium chinense* (9.81%) and *Allium cepa* (9.18%). The lowest TSS (5.3%) was recorded in genotype *Allium angulosum* (Table 2). These findings are similar to Thangasamy *et al.* (2017) [8].

Leaf area (cm²)

The leaf area of different genotypes and species under study is presented in Table 1 and 2. Leaf area varied from 8.33 cm² to 29.74. *Allium tuberosum* Rottl Ex-spr kuchaai CGN-16412(f) has significantly highest leaf area (29.74 cm²). The lowest leaf area (8.33 cm²) was recorded in *Allium macranthum* NMK-3244. Among the species *Allium cepa* showed the highest leaf area (19.53 cm²) followed by *Allium tuerosum* (14.91 cm²) and *Allium angulosum* (13.13 cm²). The lowest leaf area (9.06 cm²) was recorded in *Allium chinense* (Table 2).

Table 1: Mean performance of underutilized and cultivated *Alliums* for Physiological traits under study

Eentries	Dry matter (%)	Moisture (%)	RWC (%)	MSI (%)	TSS (%)	Total chlorophyll (g/ml)	chlorophyll 'a' (g/ml)	chlorophyll 'b' (g/ml)	Leaf area (cm ²)
<i>Allium angulosum</i> EC-328486	13.87	86.13	84.04	34.61	5.30	1.29	0.99	0.30	13.13
<i>Allium chinense</i> NG-3165	38.67	61.33	64.25	14.77	10.07	2.95	2.25	0.70	8.53
<i>Allium chinense</i> NMK-3247	41.67	58.33	62.18	0.89	9.13	1.33	1.00	0.33	9.65
<i>Allium chinense</i> NMK-3249	42.00	58.00	69.62	6.74	10.23	1.87	1.42	0.44	8.99
<i>Allium macranthum</i> NMK-3216	38.33	61.67	82.84	2.74	14.27	2.75	2.12	0.63	8.81
<i>Allium macranthum</i> NMK-3227	39.67	60.33	67.47	9.38	11.34	1.81	1.39	0.42	10.02
<i>Allium macranthum</i> NMK-3229	40.67	59.33	68.52	3.79	11.43	2.14	1.66	0.48	9.65
<i>Allium macranthum</i> NMK-3232	40.33	59.67	73.37	9.24	10.17	1.87	1.48	0.39	8.96
<i>Allium macranthum</i> NMK-3233	50.00	50.00	68.23	10.61	11.23	2.01	1.56	0.45	10.26
<i>Allium macranthum</i> NMK-3236	37.67	62.33	71.39	0.23	9.33	1.28	1.00	0.27	10.51
<i>Allium macranthum</i> NMK-3237	38.00	62.00	69.60	11.50	12.43	1.27	0.96	0.31	9.18
<i>Allium macranthum</i> NMK-3238	33.67	66.33	66.26	20.48	11.23	2.08	1.62	0.47	8.94
<i>Allium macranthum</i> NMK-3240	35.33	64.67	66.43	7.40	10.27	2.86	2.15	0.71	9.85
<i>Allium macranthum</i> NMK-3242	46.33	53.67	78.36	7.61	12.23	1.64	1.24	0.40	8.75
<i>Allium macranthum</i> NMK-3243	35.33	64.67	68.20	3.70	12.10	1.55	1.16	0.40	8.56
<i>Allium macranthum</i> NMK-3244	40.00	60.00	68.39	2.42	11.00	2.38	1.82	0.56	8.33
<i>Allium macranthum</i> NMK-3245	44.00	56.00	81.15	11.46	10.23	2.20	1.72	0.48	8.93
<i>Allium macranthum</i> NMK-3246	39.33	60.67	64.27	0.39	11.33	1.73	1.27	0.45	9.09
<i>Allium tuberosum</i> Bawang kuchaai-CGN-15749	13.07	86.93	79.43	8.11	7.17	3.73	2.89	0.84	13.78
<i>Allium tuberosum</i> CGN-16418(Flowering)	15.33	84.67	86.22	34.35	6.07	2.59	2.11	0.48	14.47

Allium tuberosum CGN-16418(Non flowering)	15.00	85.00	86.22	35.48	9.93	3.22	2.46	0.77	14.30
Allium tuberosum EC-607483	17.87	82.13	82.60	58.08	8.07	2.55	1.93	0.62	13.86
Allium tuberosum kazakhstan All-1587	14.00	86.00	85.22	24.66	5.00	3.86	2.46	0.77	16.83
Allium tuberosum MKG-84	16.67	83.33	81.54	16.37	7.83	3.38	2.60	0.78	10.42
Allium tuberosum MKG-85	17.80	82.20	84.51	15.44	7.93	3.37	2.66	0.72	10.47
Allium tuberosum MKG-88	14.47	85.53	82.77	28.45	7.13	3.39	2.60	0.78	10.34
Allium tuberosum NG-3183	15.80	84.20	72.28	39.84	6.33	7.69	5.87	1.82	15.83
Allium tuberosum NMK-3207	17.67	82.33	82.22	2.90	9.87	5.68	4.44	1.24	12.16
Allium tuberosum NMK-3211	15.00	85.00	85.58	30.86	5.97	3.98	3.13	0.85	13.13
Allium tuberosum NMK-3214(Non Flowering)	15.80	84.20	84.54	43.43	7.17	3.20	2.52	0.68	11.94
Allium tuberosum NMK-3228	14.47	85.53	86.55	45.40	6.33	3.74	2.92	0.82	12.08
Allium tuberosum NMK-3229	15.47	84.53	85.20	8.48	8.00	2.86	2.18	0.68	12.61
Allium tuberosum NMK-3231	16.40	83.60	78.50	15.33	8.77	2.77	2.16	0.61	11.74
Allium tuberosum Rottl Ex-spr kuchaai CGN-16373	18.33	81.67	84.71	4.44	5.43	6.75	5.33	1.42	14.23
Allium tuberosum Rottl Ex-spr kuchaai CGN-16412(f)	15.13	84.87	90.64	1.78	9.17	3.88	3.02	0.86	29.74
Allium tuberosum zimmu	15.40	84.60	82.54	5.64	8.17	2.49	1.93	0.57	12.20
Allium tuerosum NMK-3219	12.87	87.13	87.26	8.40	6.23	5.43	4.27	1.16	13.20
Bhima Kiran	15.60	84.40	57.70	5.40	12.17	4.19	3.14	1.04	20.45
Bhima Shakti	15.80	84.20	62.57	25.18	10.20	3.84	2.91	0.93	21.15
Bhima Shweta	15.07	84.93	55.90	5.00	5.17	3.32	2.60	0.72	17.00
Mean	25.95	74.05	75.98	15.52	9.04	3.02	2.32	0.68	12.80
C.D. 5%	11.62	11.62	0.70	6.76	0.46	0.40	0.23	0.09	17.80
C.V.	27.55	9.65	0.56	26.79	3.14	8.16	6.19	7.73	85.89
S.E.	4.13	4.13	0.25	2.40	0.16	0.14	0.08	0.03	6.35

Table 2: Mean values of Physiological traits in *Allium* species under study

Species	Dry matter (%)	Moisture (%)	RWC (%)	MSI (%)	TSS (%)	Total chlorophyll (g/ml)	Chlorophyll 'a' (g/ml)	Chlorophyll 'b' (g/g/ml)	Leaf area (sq/cm)
<i>Allium angulosum</i>	13.87	86.13	84.04	34.61	5.3	1.29	0.99	0.3	13.13
<i>Allium chinense</i>	40.78	59.22	65.35	7.47	9.81	2.05	1.56	0.49	9.06
<i>Allium macaranthum</i>	39.9	60.1	71.03	7.21	11.33	1.97	1.51	0.46	9.28
<i>Allium tuberosum</i>	15.61	84.39	83.61	22.5	7.6	3.92	3.03	0.87	14.91
<i>Allium cepa</i>	15.40	84.51	58.72	11.86	9.18	3.78	2.89	0.90	19.53
C D (5%)	11.62	11.62	0.7	6.76	0.46	0.4	0.23	0.09	17.8
C V	27.55	9.65	0.56	26.79	3.14	8.16	6.19	7.73	85.89
SE	4.13	4.13	0.25	2.4	0.16	0.14	0.08	0.03	6.35

Conclusion

The present study will help to know about physiological and biochemical differentiation of underutilized Alliums and cultivated Alliums and it will possible to identify genetic diversity among the genotype as well as different levels of biochemical and physiological component. These genotypes can be used in further breeding programme as a source against biotic and abiotic stresses. This research finding could be useful to develop a food-based strategy to increase the bioavailability of biochemical compound, physiological compound trace minerals, vitamin, micronutrients and therefore contributes to the benefit of human health. Regular inclusion of Allium species in meals, especially in powdery form can also be a good opportunity to enhance micronutrient supply of the diet of low income earners which form majority of the society.

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