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

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Abstract
Onion (Allium cepa L.), a member of Aliiaceae 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, Raigunagar, 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 fulfill 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, Rajurunagar, Pune, Maharashtra, during rabi season of 2017-18. A total of 40 genotypes of each Allium accession were cultivated in three replicates, each in a plot of 1.0 x 2.0 m with distance of 25 x 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) [3].

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 (665 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 65ºC. 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 x 1000, where V is volume of extract and W is dry weight of sample.

Total chlorophyll content (mg gDW-1) = [20.2 x A645 + 8.02 x A663] x (V/ W) x 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 80ºC 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-

RWC (%) = [(Fresh weight – Dry weight) / (Turgid weight – Dry weight)] * 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 40ºC 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 100ºC 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-

MSI (%) = [1-C1/C2] * 100

Where,
C1=Electrical conductivity of sample at 40ºC
C2=Electrical conductivity of sample at 100ºC

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 60ºC 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.

DM (%) = [Dry weight of plant / Fresh weight of plant] * 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 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 angulosus. 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) and Allium chinense (2.05 g/ml). The lowest total chlorophyll content (1.31g/ml) was recorded in Allium angulosus (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 kuchai 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 ceca (Table 2) Relative water content in underutilized Alliums was significantly higher compared to Allium ceca (Bhima Shakti, Bhima Kiran). This finding is in close proximity with the results of Khalid et al. (2014) [2].

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 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 ceca (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 ceca (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 the Table 1 and 2. Leaf area varied from 8.33 cm² to 29.74. Allium tuberosum Rott Ex-spr kuchai 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 ceca 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

| Genotypes                         | Dry matter (%) | Moisture (%) | RWC (%) | TSS (%) | Total chlorophyll (g/ml) | chlorophyll ‘a’ (g/ml) | chlorophyll ‘b’ (g/ml) | Leaf area (cm²) |
|-----------------------------------|----------------|--------------|---------|---------|--------------------------|------------------------|------------------------|-----------------|
| Allium angulosum EC-328486        | 13.87          | 86.13        | 84.04   | 34.61   | 5.30                     | 1.29                   | 0.99                   | 0.30            | 13.13          |
| Allium chinense NG-3165           | 38.67          | 61.33        | 64.25   | 14.77   | 10.07                    | 2.95                   | 2.25                   | 0.70            | 8.53           |
| Allium chinense NMK-3247          | 41.67          | 58.33        | 62.18   | 0.89    | 9.13                     | 1.33                   | 1.00                   | 0.33            | 7.95           |
| Allium chinense NMK-3249          | 42.00          | 58.00        | 69.62   | 6.74    | 10.23                    | 1.87                   | 1.42                   | 0.44            | 8.99           |
| Allium macranthum NMK-3216        | 38.33          | 61.67        | 82.84   | 2.74    | 14.27                    | 2.75                   | 2.12                   | 0.63            | 8.81           |
| Allium macranthum NMK-3227        | 39.67          | 60.33        | 67.47   | 9.38    | 11.34                    | 1.81                   | 1.39                   | 0.42            | 10.02          |
| Allium macranthum NMK-3229        | 40.67          | 59.33        | 68.52   | 3.79    | 11.43                    | 2.14                   | 1.66                   | 0.48            | 9.65           |
| Allium macranthum NMK-3232        | 40.33          | 59.67        | 73.37   | 9.24    | 10.17                    | 1.87                   | 1.48                   | 0.39            | 8.96           |
| Allium macranthum NMK-3233        | 50.00          | 50.00        | 68.23   | 10.61   | 11.23                    | 2.01                   | 1.56                   | 0.45            | 10.26          |
| Allium macranthum NMK-3236        | 37.67          | 62.33        | 71.39   | 0.23    | 9.33                     | 1.28                   | 1.00                   | 0.27            | 10.51          |
| Allium macranthum NMK-3237        | 38.00          | 62.00        | 69.60   | 11.50   | 12.43                    | 1.27                   | 0.96                   | 0.31            | 9.18           |
| Allium macranthum NMK-3238        | 33.67          | 66.33        | 66.26   | 20.48   | 11.23                    | 2.08                   | 1.62                   | 0.47            | 8.94           |
| Allium macranthum NMK-3240        | 35.33          | 64.67        | 66.43   | 7.40    | 10.27                    | 2.86                   | 2.15                   | 0.71            | 9.85           |
| Allium macranthum NMK-3242        | 46.33          | 53.67        | 78.36   | 7.61    | 12.23                    | 1.64                   | 1.24                   | 0.40            | 8.75           |
| Allium macranthum NMK-3243        | 35.33          | 64.67        | 68.20   | 3.70    | 12.10                    | 1.55                   | 1.16                   | 0.40            | 8.56           |
| Allium macranthum NMK-3244        | 40.00          | 60.00        | 68.39   | 2.42    | 11.00                    | 2.38                   | 1.82                   | 0.56            | 8.33           |
| Allium macranthum NMK-3245        | 44.00          | 56.00        | 81.15   | 11.46   | 10.23                    | 2.20                   | 1.72                   | 0.48            | 8.93           |
| Allium macranthum NMK-3246        | 39.33          | 60.00        | 64.27   | 0.39    | 11.33                    | 1.73                   | 1.27                   | 0.45            | 9.09           |
| Allium tuberosum Bawang kuchaai CGN-15749 | 13.07         | 86.93        | 79.43   | 8.11    | 7.17                     | 3.73                   | 2.89                   | 0.84            | 13.78          |
| Allium tuberosum CGN-16418(Flowering) | 15.33         | 84.67        | 86.22   | 34.35   | 6.07                     | 2.59                   | 2.11                   | 0.48            | 14.47          |
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 powdered 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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