

## ORIGINAL ARTICLE

# Evaluation of morphological and physiological parameters during drought stress in Barnyard millet growing in Kumaun region of Himalayan

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### ABSTRACT

Drought stress is one of the major abiotic stress challenges in agriculture sector which limits the growth of plants and thus affected net productivity. Seeds of our varieties of barnyard millet viz VL29, VL207, VL172 and VL181 were subjected to drought stress by PEG-6000 in liquid MS medium with different osmotic potential 5% w/v (-0.5MPa), 10% w/v (-0.15MPa), 15% w/v (-0.30MPa) and 20% w/v (-0.49MPa). Morphological analysis was recorded after 19<sup>th</sup> day and 26<sup>th</sup> day of culture. Minimum and minimum shoot and root length root to shoot ratio, and seed vigor index was observed in variety VL172 and variety VL29 on both the days respectively. Germination index was observed upto 12<sup>th</sup> day of the culture and found the variety VL172 showed maximum germination index and minimum in variety VL29. Analysis of fresh weight, dry weight, RWC, moisture percent and electrolyte leakage in 12 days old seedling were imposed to various concentration of PEG and evaluated every 24 hrs up to 7 days. Relative water content (RWC), moisture content, fresh weight, dry weight and electrolyte leakage was maximum in variety VL172 and minimum in variety VL29. Percent decreased in total chlorophyll and increased in Chl a/b ratio found in variety VL172 and percent decreased in total chlorophyll was maximum in variety VL29 with minimum increased percent in chl a/b ratio in variety VL29. Therefore variety VL172 found to be tolerant and variety VL29 was susceptible to drought stress among the varieties.

**Keywords** Barnyard millet. Morphological parameters. Physiological parameters. Drought. PEG

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### INTRODUCTION

India is the largest producer of millets and there grain has been staple food for sustaining livelihood of the millions of the poorest and rural people [1, 2, 3]. Barnyard millet is a common weed of temperate and warm regions mostly cultivated in China, Korea and Japan. However it is also extensively grown in India from Kashmir to Sikkim in the north and to Tamil Nadu in the south and commonly known as "sawa" [4]. The world total production of millet grains in year 2010 was 762,712 metric tonnes and the top producer was India with an annual production of 334,500 tones contributing 43.85% of the world production [5]. In India North-Eastern Terai Region of Uttar Pradesh playing a major role for producing high amount of millets [6]. Barnyard millet (*Echinochloa frumentacea*) is grown in 0.28 million ha land having a productivity of 8.63 quintal/ha in India [7]. Being hardy and robust crops, millets can resist and survive harsh climatic conditions [8]. The low productivity of barnyard millet is caused by due to drought and grown in temperate northern Himalayan areas up to altitude of 3000 meters. To increase the productivity of barnyard millet it is essentially required to study the morphological and physiological response in presence of drought of the varieties grown in the Himalayan region. Drought is a multidimensional abiotic stress factors that affects the plants by many ways by reducing plant growth, metabolism and yield [9]. Drought induces the stomata closure, thereby reduction in CO<sub>2</sub> production which increase in the production of ROS in different cellular and sub cellular compartments [10]. Plants undergo several mechanisms to combat to increase level of ROS, some changes morphological like increasing root hairs. There are also physiological changes like alteration in carbon partitioning, closure of stomata, osmotic adjustment [11]. Osmotic adjustment help the plants in maintaining high relative water content at low water potential [12]. Relative water content reflects the balance between water supply to leaf tissue and transpiration rate [13]. The level of tolerance to drought stress varies from plant

species to species and even in single species. In our study among the four different varieties of barnyard millet to the drought tolerant ability also showed huge variation in the level of adaptability to drought stress. As no literature available on the drought tolerant ability of barnyard millet, therefore to understand drought tolerant mechanism of barnyard millet at morphological and physiological level screening was conducted and screen out tolerant and susceptible variety among these four varieties VL172, VL207, VL29, and VL181 of barnyard millet grown in Kumaun region of Himalaya to understand the mechanism for drought resistance.

## MATERIAL AND METHODS

**Plant material and growth conditions-** Seeds of four varieties of barnyard millet *viz*, VL29, VL207, VL172, VL181 were procured from Vivekananda Parvatiya Krishi Anusandhan Sansthan (VPKAS) Almorah, Uttarakhand, India. All the experimental studies were carried *in vitro* in MS medium. The germinating seedlings of barnyard millets were exposed for drought with PEG-6000 in MS medium. Before inoculation, seeds of Barnyard millet varieties (VL207, VL29, VL172 and VL181) were washed with distilled water followed by treatment of Tween-20 detergent for 20 min and again thoroughly washed under running tap water for 20 min to remove all traces of detergent. The seeds were treated with 0.1% w/v bavastin for 1 min, 70% v/v ethanol for 2 min and 0.1% w/v HgCl<sub>2</sub> for 1 min. Each steps seeds were washed with autoclave distilled water. These seeds were blot dried in filter paper and ten seeds per bottle were placed on liquid MS medium with the help of sterilized forceps. All the steps of inoculation were performed in the laminar air flow under aseptic conditions. After inoculation bottles were kept in tissue culture chamber for 12 days at temp of 25±1°C and a relative humidity of 70% under illumination with 40 W fluorescent tubes having a photon flux density of approximately 21,500 lux with a 16h/8 day/night cycle. Twelve days old seedlings were treated with different concentration of PEG 6000 i.e. 5% w/v (-0.5MPa), 10% w/v (-0.15MPa), 15% w/v (-0.30MPa) and 20% w/v (-0.49MPa) in liquid MS medium. To study long term effect of drought stress on morphological aspects like on shoot, root length and their ratios, seed vigor index, seeds were grown in liquid MS medium containing different PEG 6000 concentration i.e. 5% w/v (-0.5MPa), 10% w/v (-0.15MPa), 15% w/v (-0.30MPa) and 20% w/v (-0.49MPa) and plant material were collected on 19<sup>th</sup> days and 26<sup>th</sup> days.

**Morphological analysis-** Shoot length and root length were recorded on 19<sup>th</sup> and 26<sup>th</sup> days in control as well as drought treated plants. Root to shoot ratio was calculated by root length divided by shoot length. Vigor index was calculated by Dhindwal *et al.* [14]. Seeds were grown in MS medium containing different concentrations of PEG and seeds were left to attained normal seedling stage which was up to 12 days. After 12 days seeds remain in same MS medium to grow for one week so that parameters would be taken on 19<sup>th</sup> day and 26<sup>th</sup> day from the day of inoculation to study the potential of all four varieties that how they can withstand drought condition up to long period of duration.

Vigor index = (mean root length + mean shoot length) × % germination

Germination index calculated from the following formulas by official seed analysis AOSA [15]. Seeds of all the four varieties were germinated in liquid MS basal media containing different concentration of PEG 6000 i.e. 5% w/v (-0.5MPa), 10% w/v (-0.15MPa), 15% w/v (-0.30MPa) and 20% w/v (-0.49MPa). Germination count started from the 1<sup>st</sup> day up to 12<sup>th</sup> day (as its attained seedling stage up to 12<sup>th</sup> day).

GI = No. of germinated seeds / Days of first count + No. of germinated seeds/ Days of second count+...+ No. of germinated seeds/ days of final count

**Physiological analysis-** The fresh weight was calculated in 12 days old seedlings were taken immediately after the 24 hrs of treatment with four different concentration of PEG along with the 12 days old control plants. Similarly the dry weight of 12 days old seedlings were taken immediately after the 24 hrs of treatment with four different concentration of PEG along with the 12 days old control plants after oven drying at 64°C until obtained constant weight.

Relative water content was determined by Weatherley [16]. Leaf disc (1g) was prepared from the control and drought treated plants. Discs were immersed in 10 ml of water and kept for 3 hrs at room temperature. Turgor weight was measured after blot drying the samples. The samples were then oven dried at 65°C until obtained constant weight. Dry weight of samples was taken and RWC was calculated using following formula.

Relative water content (%) = (F.W-D.W / T.W- D.W) × 100

Moisture content of early seedling of all four genotype which were grown in liquid MS-basal media for 12 days and then changed the media containing four different concentrations of PEG. Moisture content was measured in 12 days old seedling on 1<sup>st</sup> day, 3<sup>rd</sup> day, 5<sup>th</sup> day and 7<sup>th</sup> day.

Moisture content = [(F.W - D.W) / F.W] × 100

Total chlorophyll content of control and drought treated plants was determined by using method described by Hiscox and Israelstam [17]. Ratio of Chlorophyll a/b was also calculated. Chlorophyll stability index was calculated by dividing the chlorophyll content of treated plant by the chlorophyll content of control plant expressed in percentage Deshmukh *et al.* [18].

Electrolyte leakage (EL) was estimated according to Dionisio-Sese and Tobita [19]. One gram of fresh leaf samples were washed with triple distilled water and were cut into small pieces (~1 cm segments) and suspended in test tubes containing 10 ml of de-ionized water. Tubes were incubated in a water bath at 32°C for 2 hrs. After incubation, electrical conductivity (EC1) of the bathing solution was recorded. These samples were then autoclaved at 121°C for 20 min to completely killed the tissues and release the electrolytes. Samples were then cooled to 25°C, and final electrical conductivity (EC2) was measured. The percent leakage of electrolytes was calculated using the formula.

Percent Electrolyte Leakage =  $(EC1/EC2) \times 100$

**Statistical analysis-** The data presented are mean values  $\pm$ SE. Measurements were performed on the three replicates for each treatment ( $n=3$ ). Data subjected to analysis of variance (ANOVA), using SPSS16 (Stastical Package for the social science). Mean were separated by the Duncan when analysis of variance (ANOVA) was significant ( $P<0.05$ ). Correlation analysis (bivariate) was also performed to determine the relationship between electrolyte leakage and RWC.

## RESULTS AND DISCUSSION

**Effect of drought on Morphological parameters-** An abiotic stress is very complex phenomenon because of complicated of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development [20]. A significant decreased in shoot length and root length was observed in all the four varieties (Fig. 1a). In variety VL29 no germination was observed at 20% w/v PEG (-0.49MPa) concentration among the others three varieties. So that observations was made to observed at 15% w/v PEG (-0.30MPa) on 19<sup>th</sup> day and 26<sup>th</sup> day of treated plants. On 26<sup>th</sup> day treated plant minimum percent reduction in shoot length 37.93% was observed in variety VL172 compared to control of their respective day at maximum level of PEG stress (20%), followed by variety VL207 (38.15%), VL181(47.97%) and VL29(48.65%). Similar pattern was observed in root length percent decreased among the varieties, minimum percent decreased was in variety with 54.66% compared to control at 20% PEG, followed by VL207 (58.10%), VL181 (62.44%) and maximum was observed in variety VL29 (73.85%) (Fig. 1b). Decreased in shoot length was related to less used to metabolites so that plants can synthesized more osmolytes and their adjustment which play an important role in drought and others stress conditions [21]. The response to drought varies among different varieties. The reduction in plant height was associated with a decline in the cell enlargement and more leaf senescence in *A.esculentus* under water stress [22]. The “balanced growth” hypothesis [23] suggested that in some plants there is increased root length as compared to shoot because it enables the plants to taken out more water from the soil. There by it improve plant hydraulic efficiency under mild or moderate drought stress because of increased root to leaf surface resulted in continued production of new root tips which helps the plant to extract water from deep layer of soil and supporting the shoot livelihood. But it has been observed that under severe water deficits, limited root growth may occur because of very low soil water availability and high soil impedance [24, 25, 26, 27]. Similar kind of observation was reported in our result of root length measurements where root length were found to be not increased as compared to shoot length as the seedling were grown in MS medium containing different PEG concentrations, may causes the severe drought effects on the plants so that it causes root length not to increase as compared to shoot length. Thus Root to shoot ratio is a very important criteria to enable the plants to resist the drought conditions by extracting the water through their roots. In our results maximum root to shoot ratio was observed in variety VL172 and minimum was found in variety VL29 at 15% PEG concentration on 19<sup>th</sup> and 26<sup>th</sup> day treated plants. On maximum day of stress that on 26<sup>th</sup> day, maximum root to shoot ratio was observed in variety VL172 (0.76), however in two varieties VL181 and VL207 (0.24) showed similar level of ratios but in variety VL29 (0.17) minimum ratio was observed (Fig. 1c). From above result it was correlated that variety VL172 showed better compatibility among the others three varieties to drought conditions in respect to shoot length, root length and root to shoot ratio. In maize cultivar root to shoot ratio was observed to decreased when increased PEG-6000 concentration [28]. Different accessions of sorghum (*Sorghum bicolor L.*) were evaluated for their ability to drought tolerance at seedling stage. It has been observed that all seedling parameters got negatively affected by water deficit [29]. In tomato germplasm seedling the reduction of shoot and root length were observed which were grown in PEG 6000 [30].

Assessment of seed vigor has many important factors to the monitoring of seed physiological potential during different phases of seed production. Seed vigor parameters also assess to evaluate directly or indirectly seed metabolic state or identify seed tolerance to specific stress(es). There are various factors which affect the seed physiological potential include germination (viability) and vigor, which direct the ability of seeds to state their vital functions under both favorable and unfavorable environmental conditions [31]. Study showed that higher vigor that occurred in larger seed is due to the larger food reserves in these seeds [32]. Study was observed on 19<sup>th</sup> day and 26<sup>th</sup> day at 15% PEG concentration as in variety VL29 no growth was observed at 20% concentration (Fig.1d). On long day of stress condition that is on 26<sup>th</sup> day, minimum percent reduction was recorded in variety VL172 (49.83%) which was followed by variety VL207 (50.98%), variety VL181 (66.69%) and maximum percent reduction compared to control was recorded in variety VL29 (69.05%) at 15% PEG concentration on 26<sup>th</sup> day of stress plants. This result indicated that variety VL172 having higher ability to survive in drought condition. In two variety of pearl millet germination percentage and seedling vigor index (SVI) decreased with increasing salinity level [33]. In study of three sorghum (*Sorghum bicolor* L.) genotypes showed that severe reduction in germination percentage and germination, vigor index by low temperature stress [34]. Germination and early growth were affected by drought stress imposed through PEG 6000 which was studied in maize genotypes [35].

Germination index interprets for germination capacity of seeds under any treatment [36]. Seed germination is negatively affected by stress conditions. Seed germination is an important and vulnerable stage in the life cycle of seeds and determines seedling establishment and plant growth [37]. Drought stress affect the seed metabolism and altered the level of endogenous hormones, thereby reducing energy production for the sufficient germination of seed and early seedling survival [38, 39, 40]. In our finding minimum percent reduction in germination index was observed in variety VL172 (27.87%), followed by variety VL207 (29.80%), then in variety VL181 (31.73%) and maximum percent reduction was observed in variety VL29 (41.93 %) (Fig.1e). Variety VL172 showed higher germination index ability in drought conditions than others varieties. *In vivo* study on wheat cultivar to evaluate the germination index in relation to increasing percent of PEG 6000 [41]. Similar study carried out in chick pea where reduction in germination rate with the increase PEG was observed [42]. Water stress affect at various level of seed at germination stage by delaying or reduced or totally stop the germination. However, if seed attained the critical level of hydration it will lead to full seed germination. However, fails to attain that particular level it lead to complete inhibition of seed germination [43, 44].

**Effect of drought on physiological parameters-** Fresh weight and dry weight of seedling showed no much noticeable variations as the level of PEG concentration increased. There was approximately same fresh and dry weight was recorded in all the variety. However minimum percent reduction in fresh weight as compared to control was observed in variety VL172 (29.04%), followed by variety VL207 (32.01%), then in variety VL181 (32.21%) and maximum percent reduction was observed in variety VL29 (33.20%) on the 7<sup>th</sup> day and at 20% PEG concentration (Fig. 2a, b, c, d). In dry weight minimum percent reduction was observed in variety VL172 (24.54%), followed by variety VL207 (24.66%), then in variety VL181 (25.35%) and minimum percent reduction in variety VL29 (25.68%) on the 7<sup>th</sup> day at 20% PEG concentration (Fig. 3a, b, c, d). Variety VL172 showed minimum reduction in fresh weight and dry weight which resist it to drought prone situation. A common effect of water stress on crop plants is the reduction in fresh and dry weight [12]. In all the genotypes of sunflower there was observed reduction in biomass due to water stress [45]. Reduced biomass by water stress was seen in various plants like soybean [46] common bean and green gram [47], *Poncirus trifoliata* seedlings [48] and *Petroselinum crispum* [49].

Relative water content is an important parameter to assess the water status in plants; its shows the level of stability between water supply to the leaf tissue and transpiration rate [13]. Reduction in relative water content depends on plant vigor reduction and which has been examined in various plants [50]. In our finding, it was observed that very less percent reduction as compared to control in all the four varieties in stress treated plants (Fig.4a, b, c, d). It may be concluded that as barnyard millet is drought resistant plant, it resist the loss of water content in the cell through stomata closure. However, minimum percent reduction as compared to control was found in variety VL172 (6.97%), followed by in variety VL207 (7.81%), then in variety VL181 (8.92%) and maximum percent reduction was observed in variety VL29 (25.10%) on the 7<sup>th</sup> day at 20% PEG concentration. In variety VL172 higher RWC indicated it drought tolerant variety. RWC remain high in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures [51], as it correlated with our finding which indicated less decreased in dry weight which may be a reason for less decreased in RWC in barnyard millet. The effect of water stress on the wheat cultivars, showed very less reduction in RWC [52]. Drought tolerant plant species keep high RWC compared with drought-sensitive species in cultivars of sugarcane [53]. Different

varieties of sorghum showed decreased RWC under various water stress regimes [29]. A study showed that in pearl millet genotypes there was lower reduction in RWC under water stress [54].

Moisture percent in the seedling expressed in percentage, which decreased as the percent of PEG concentration was increased. In present study similar to RWC, moisture percent also not significantly decreased and not differed among the varieties (Fig. 5 a, b, c, d). In our results it was found that variety VL172 showed minimum percent decreased (0.70%), it was followed by variety VL207 (1.26%), then in variety VL181 (1.27%) which was approximately similar to VL207 and maximum percent reduction was observed in variety VL29 (1.65%) on 7<sup>th</sup> day at 20% PEG concentration. As millet considered as drought tolerant cereal which make it enable to minimum loss of moisture content. Hence it becomes clear that in various concentration of PEG the varieties of barnyard millet resist the loss of moisture. However minimum moisture loss was observed in variety VL172.

Chlorophyll content decreased as the level of drought stress increased because it greatly affects the photosynthesis because closures of the stomata and due to that less availability of CO<sub>2</sub> [55]. The decrease in Chl content has observed in several plants under drought stress [12, 56, 57, 58, 59, 60]. It indicated that minimum percent reduction in total Chl content was observed in variety VL172 (33.48%), followed by in variety VL207 (39.06%) then in variety VL181 (41.60%) and maximum was observed in variety VL29 (44.58%) (Fig. 6a, b, c, d). It was observed that in variety VL172 Chl content was less susceptible to damaged making it drought tolerant. It was found that in pearl millet decreased in chlorophyll content up to 10-30% considered as "non-lethal" under harsh drought stress [61] which was similar to our experimental finding of total chlorophyll content that also decreased up to 10-30%. Decreased in chlorophyll under stress condition due to a lowered capacity for light harvesting because the production of reactive oxygen species is mainly driven by excess energy absorption in the photosynthetic apparatus, this might be avoided by degrading the absorbing pigments [59]. It may be concluded that dehydration causes chlorophyll catabolism [62]. In wheat cultivar there was decreased in total chlorophyll was observed in drought stress [63, 64]. PEG induced drought stress imposed to plants Pigeon pea, significantly decreased chlorophyll a, chlorophyll b and total chlorophyll content [65].

It was observed that under drought stress the reduction of Chl<sub>b</sub> was greater than that of Chl<sub>a</sub>, thus, transforming the ratio in favor of Chl<sub>a</sub> [66, 67]. In our experimental finding it was observed that minimum percent reduction in Chl a was observed in variety VL172 (27.23%), followed by variety VL207(32.78%), then in variety VL181 (36.97%) and maximum was observed in variety VL29 (42.35%) (Fig. 7a, b, c, d). Similar pattern in percent reduction in Chl b was also observed among the varieties, minimum in variety VL172 (43.28%), followed by variety VL207 (45.72%), then in variety VL181 (46.88%) and maximum was observed in variety VL29 (46.94%) on the 7<sup>th</sup> day of stress at concentration of 20% PEG (Fig. 8a, b, c, d). This result showed greater reduction in Chl b than Chl a. This showed higher ratio of Chl a/b. It was also observed in our experimental finding the Chl a/b greater in variety VL172 (2.03), approximately same ratio was observed in variety VL181 (1.35) and variety VL207 (1.33) and minimum VL29 (1.25) (Fig. 9a, b, c, d). Similar result was also reported in drought tolerant wheat cultivar [68]. The total chlorophyll content decreased in pearl millet by water stress conditions was due to a significant fall in both chlorophyll a and chlorophyll b in all genotypes studied [69]. Hence concluded that discussion it was clear that variety VL172 having higher ratio of Chl a/b which makes it drought tolerant.

Chlorophyll stability index is a marker of stress tolerance ability of plants and its shows the ability of chloroplast under stress condition [70]. Chlorophyll stability index is used to measure the integrity of the membrane [71]. Maximum chlorophyll stability index was observed in our finding in variety VL172 (52, 66), followed by in variety VL207(60.94), then in variety VL181(58.40) and minimum was observed in variety VL29(55.42) on the maximum day of stress that on 7<sup>th</sup> day at 20% PEG concentration. Higher chlorophyll stability index in variety VL172 makes it drought resistant among the others. In five finger millet varieties it was observed that chlorophyll stability index found maximum in drought tolerant variety and minimum was observed in drought susceptible variety [72].

Electrolyte leakage is the method for assessing the cell membrane integrity which is usually the first targets of many plant stresses and the level of its integrity determines the drought or other stress tolerance ability of plants. The degree of cell membrane damaged by water stress may be easily estimated through measurements of electrolyte leakage from the cells [73]. It indicated that minimum percent decreased in electrolyte leakage was observed in variety VL172 (13.38), followed by variety VL207 (19.66), then in variety VL181(24.80) and maximum was observed in variety VL29(39.94) at 20% on 7<sup>th</sup> day of stress treated plant (Fig. 10 a, b, c, d). A negative correlation ( $r = -0.951$ ) was observed between relative water content and electrolyte leakage showed that barnyard millet had very less electrolyte leakage during the stress. The r-value near to -1.0 showed a perfect negative correlation (Fig.11).

The variety VL29 was found more susceptible to membrane damaged to stress. Increased electrolyte leakage and a decline of leaf RWC in water stress were established as indicators of sensitivity to drought [74]. In five finger millet varieties it was observed that electrolyte leakage found maximum in drought tolerant variety and minimum was observed in drought susceptible variety [72]. A study carried out on wheat durum where percent of electrolyte leakage increased with PEG induced treatment [73, 75].

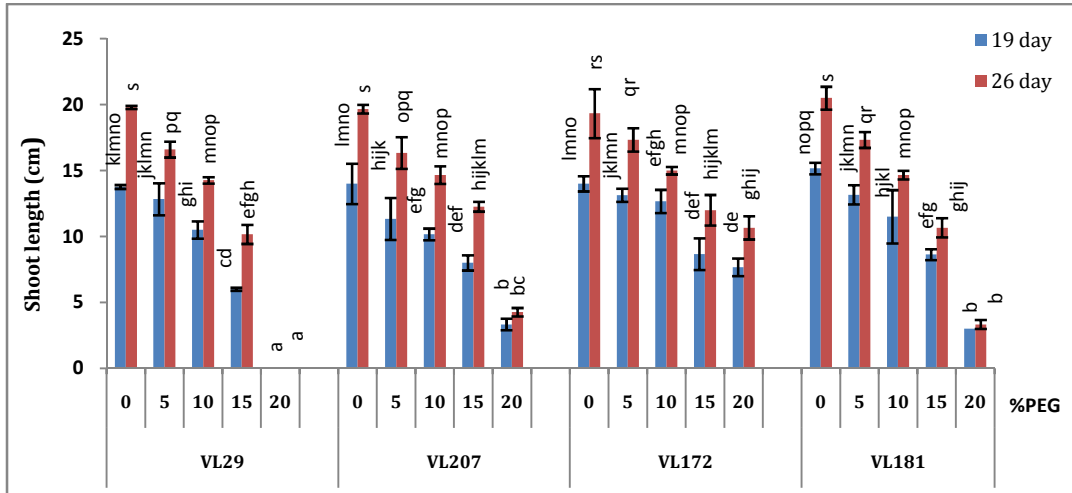


Fig. 1a

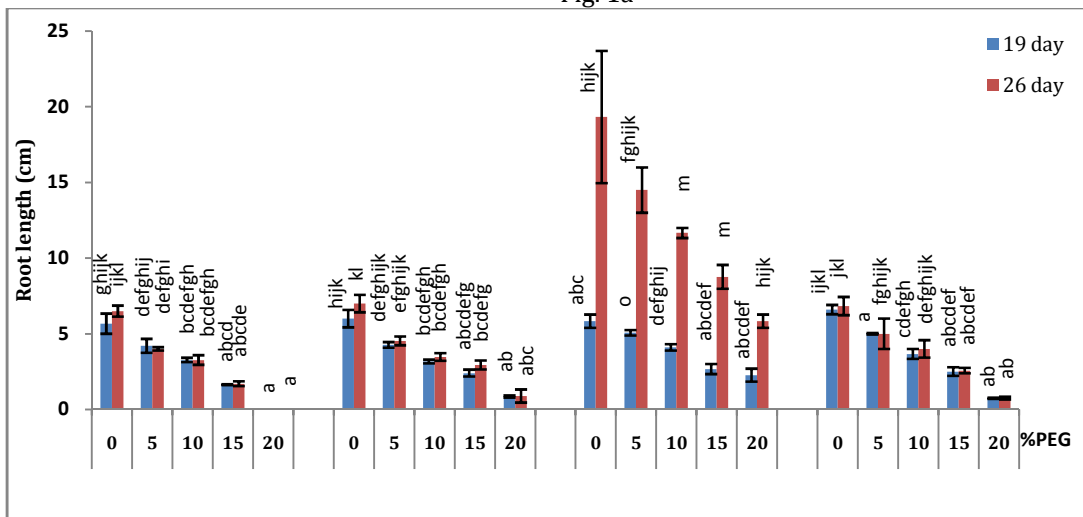


Fig1.b

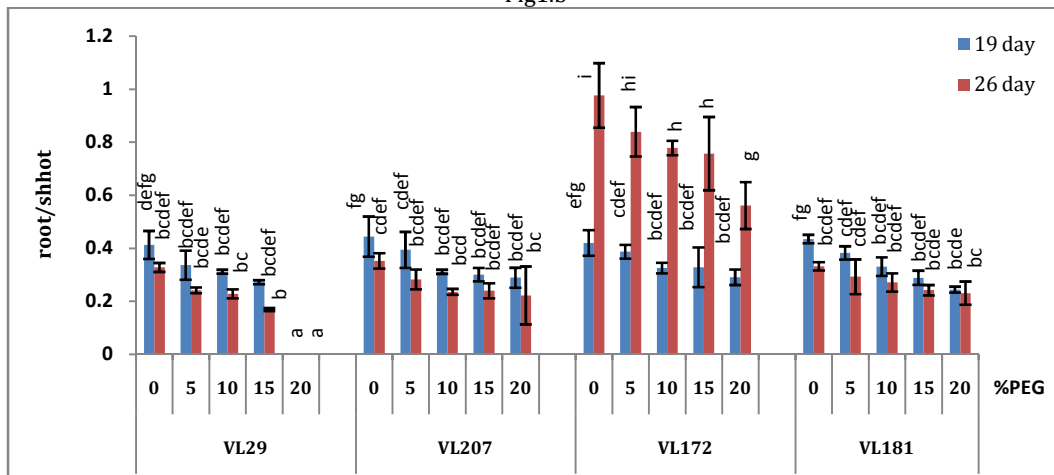


Fig 1.c

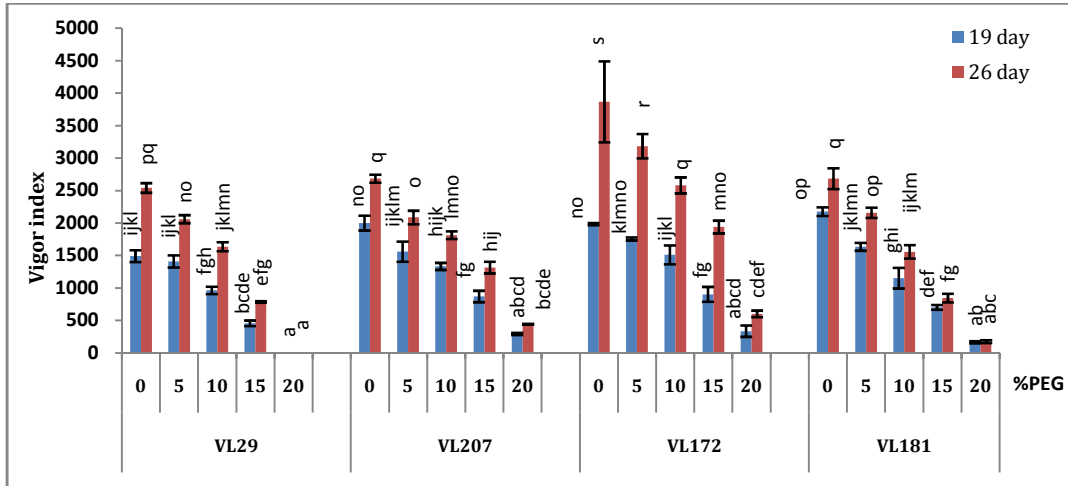


Fig1.d

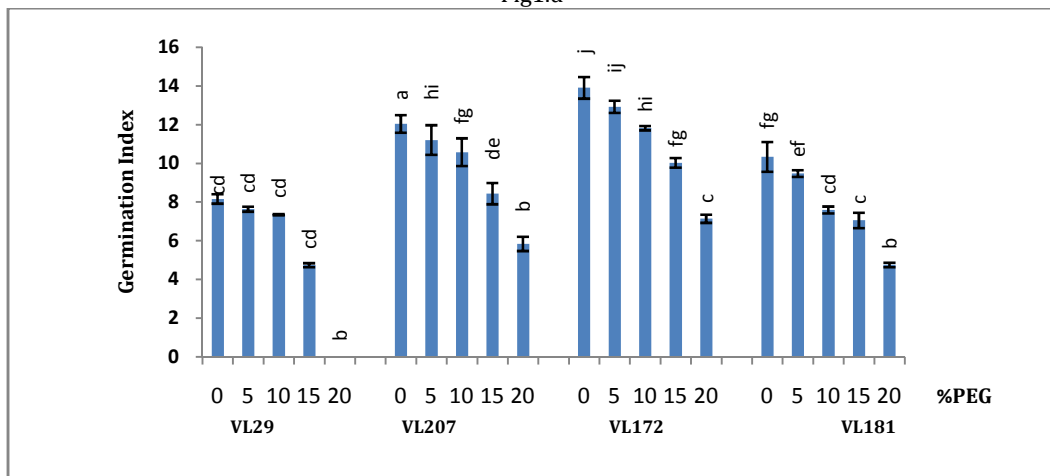


Fig1.e

**Fig.1** Morphological changes in Barnyard millet in different concentration of PEG (%) a) shoot length, (b) root length, (c) root to shoot ratio, (d) vigor index, (e) germination index. Different letters denotes significant differences (P<0.05) among four varieties in control and drought stressed plants. Line above bars represents mean ±standard error.

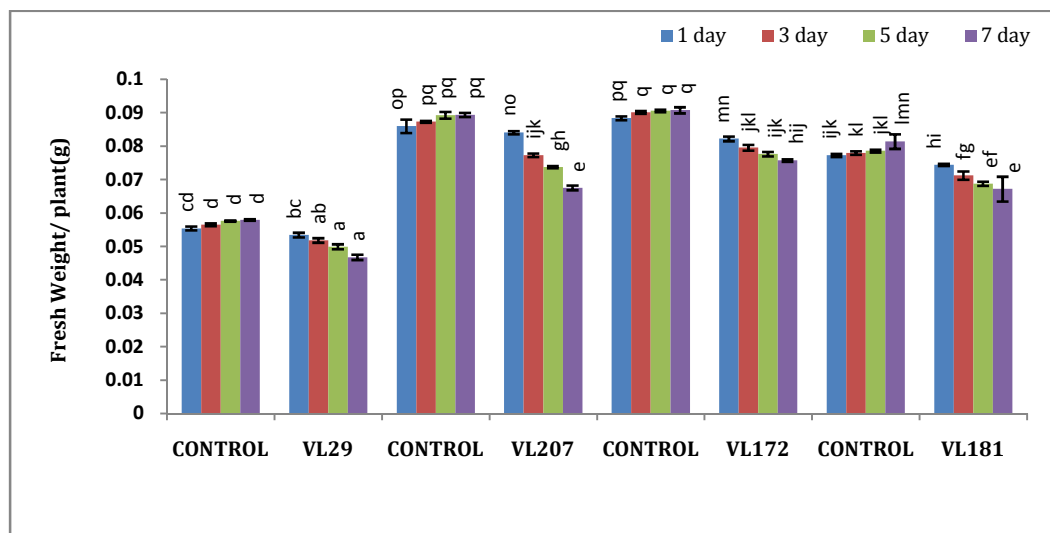


Fig 2. a

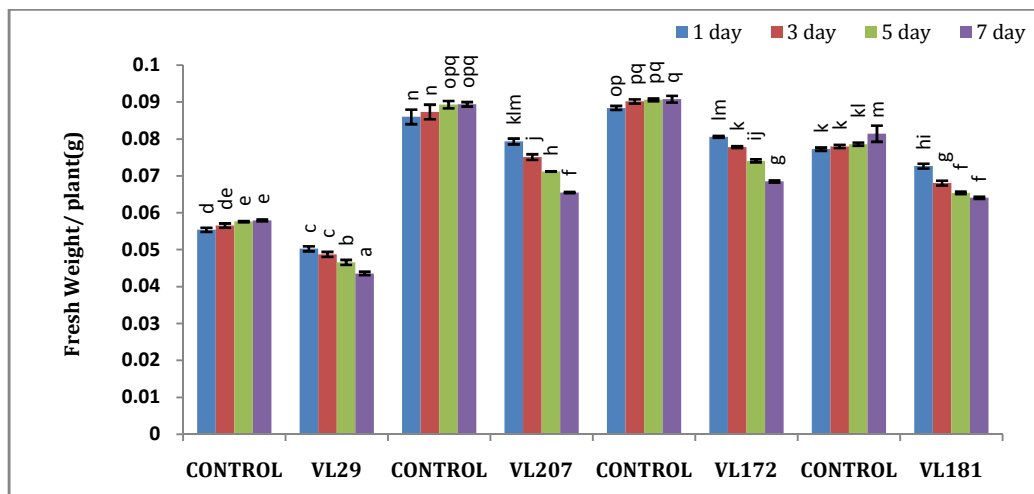


Fig 2.b

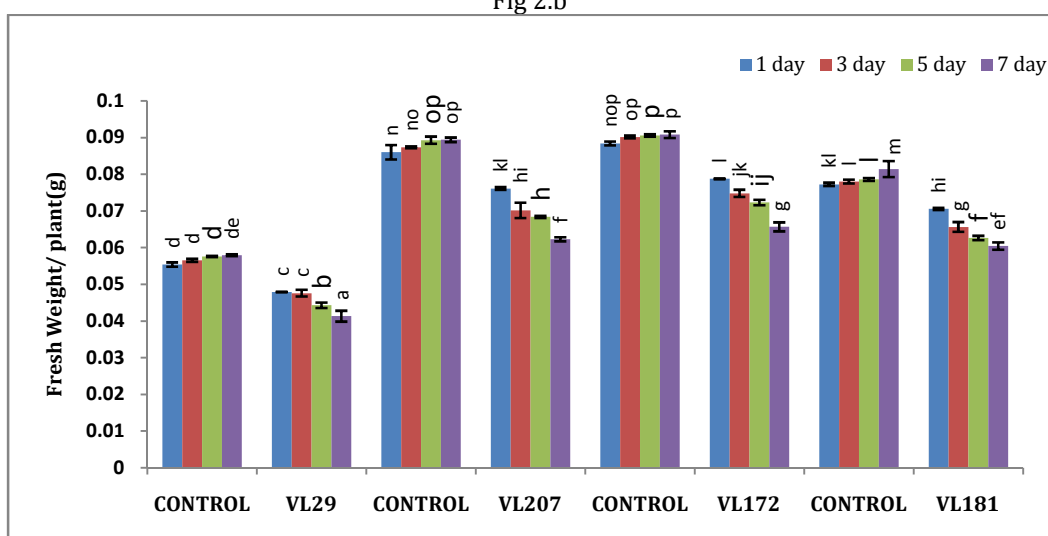


Fig 2.c

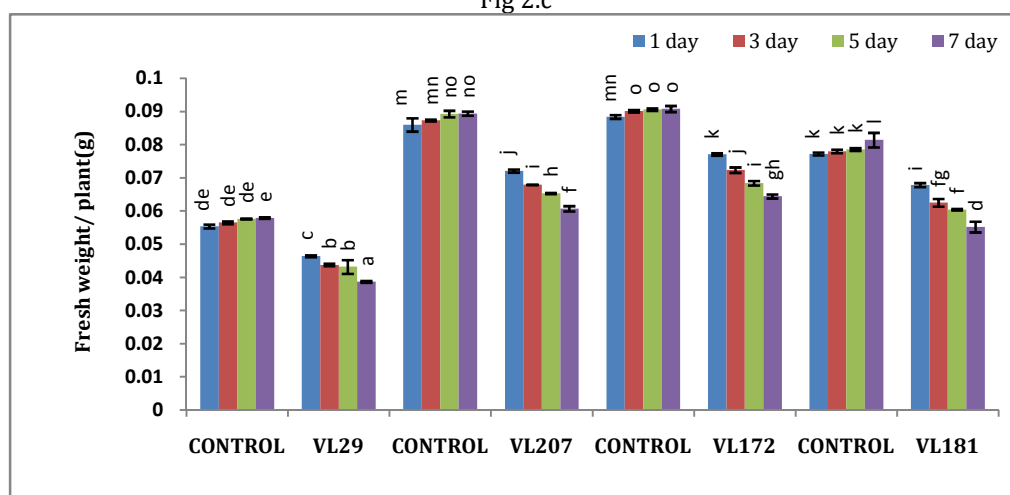


Fig 2.d

**Fig. 2** Effect of progressive drought stress by different concentration of PEG (%) on FW in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences (P<0.05) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error.



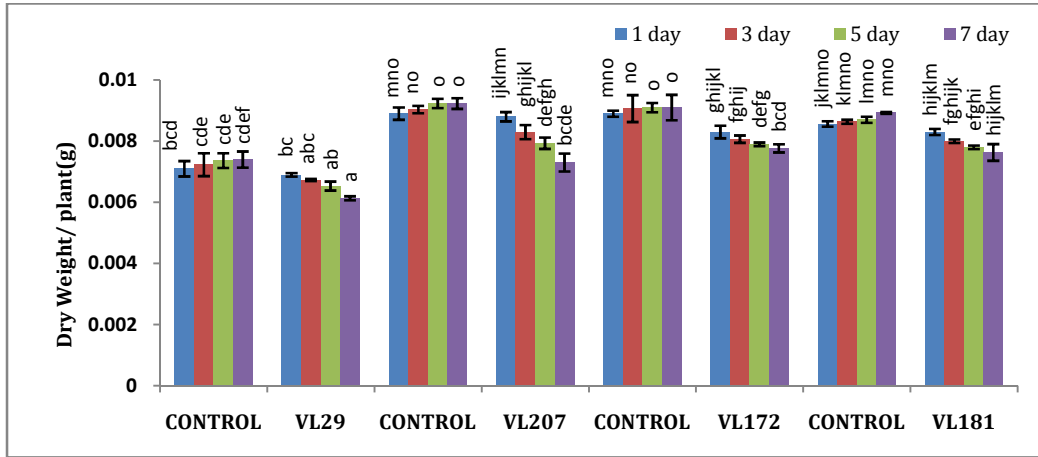


Fig 3.a

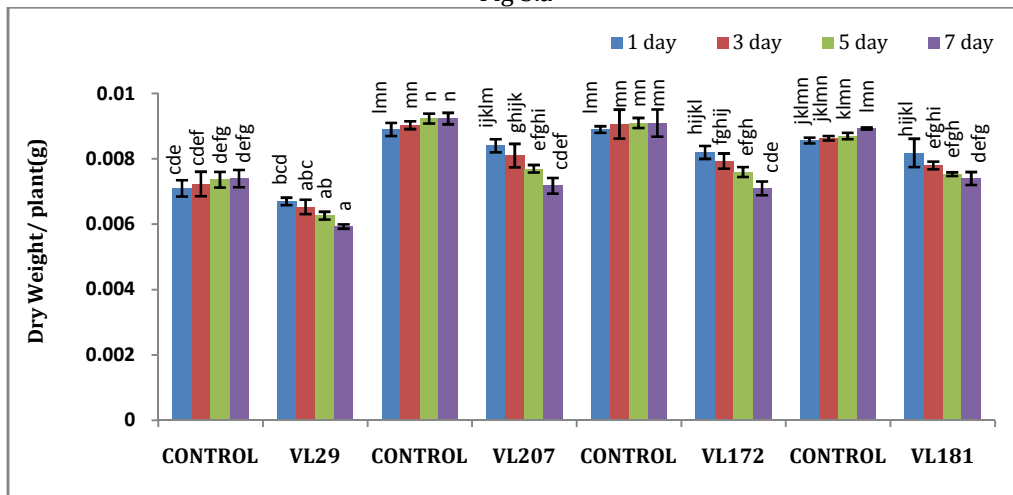


Fig.3b

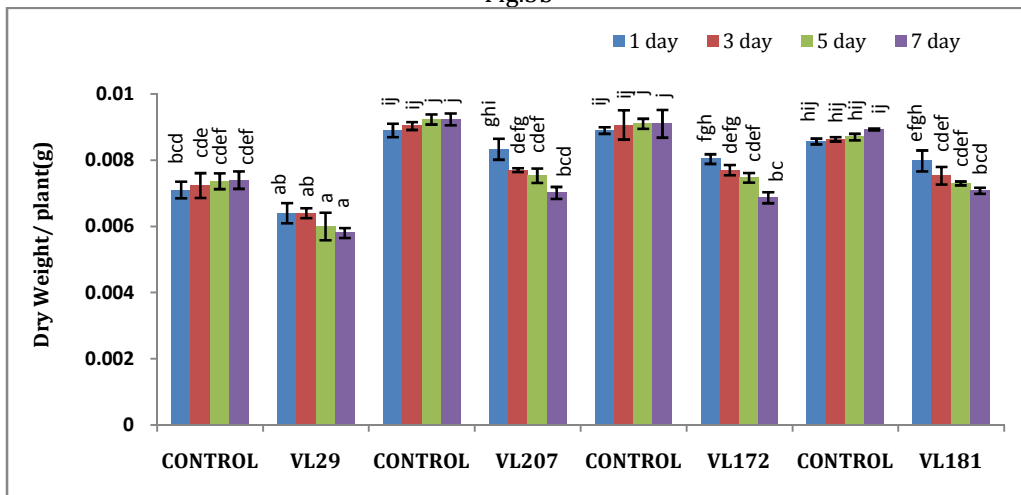


Fig 3. c

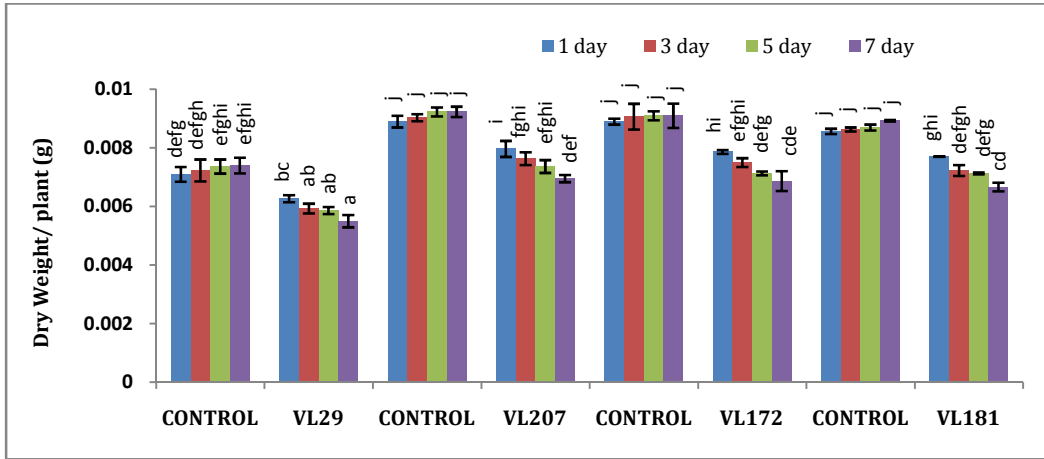


Fig 3. d.

Fig.3. Effect of progressive drought stress by different concentration of PEG (%) on DW in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences (P<0.05) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error

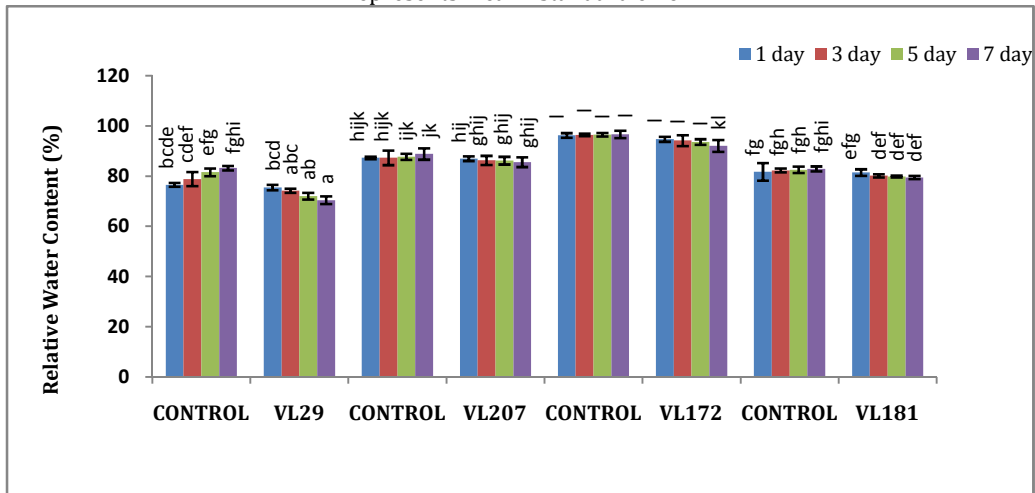


Fig 4 a

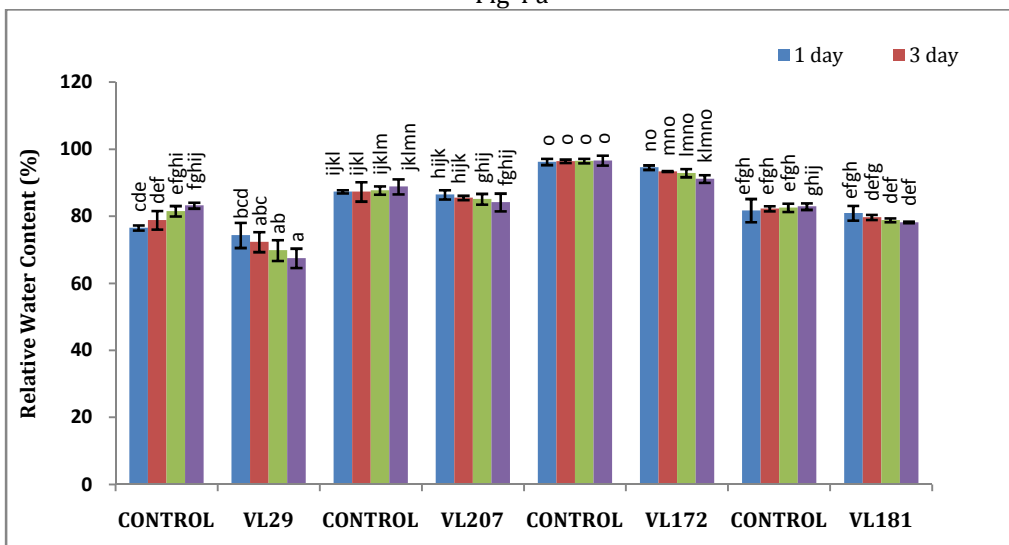


Fig 4 b

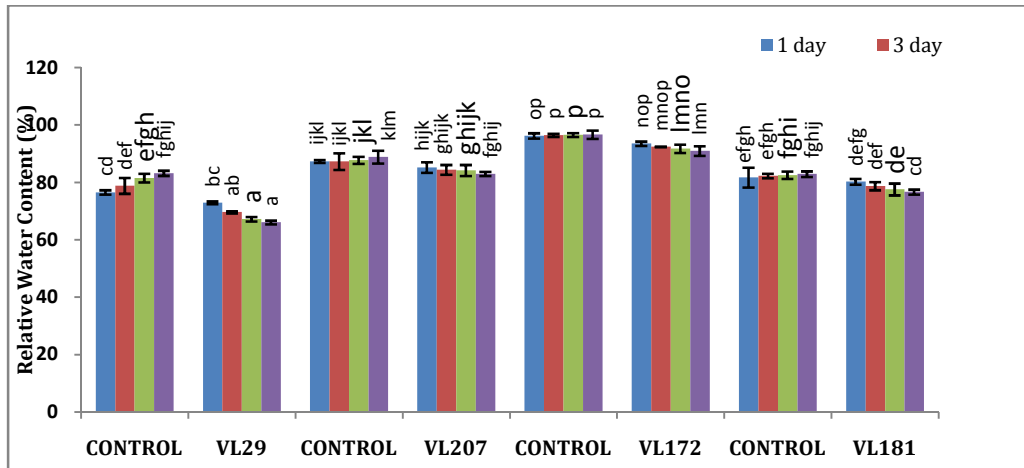


Fig 4 c

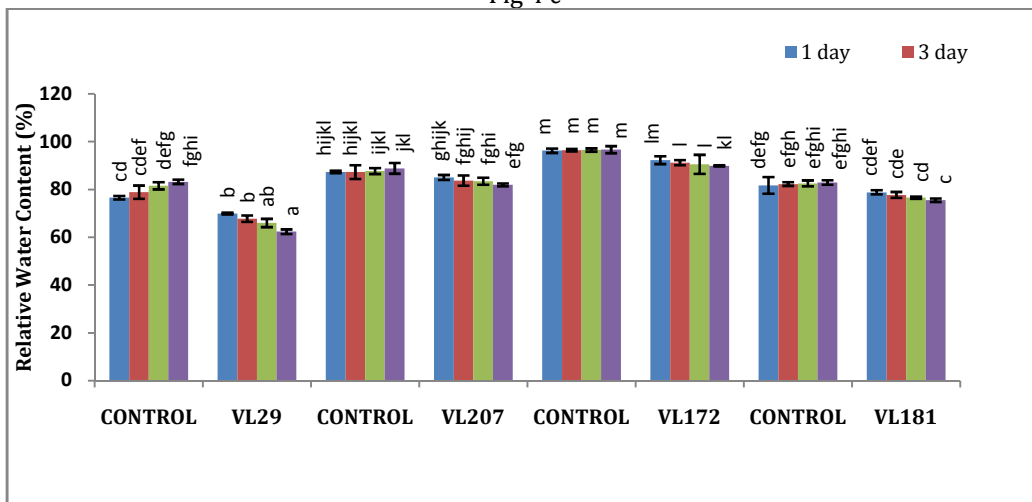


Fig 4 d

**Fig.4** Effect of progressive drought stress by different concentration of PEG (%) on RWC in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences ( $P < 0.05$ ) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error.

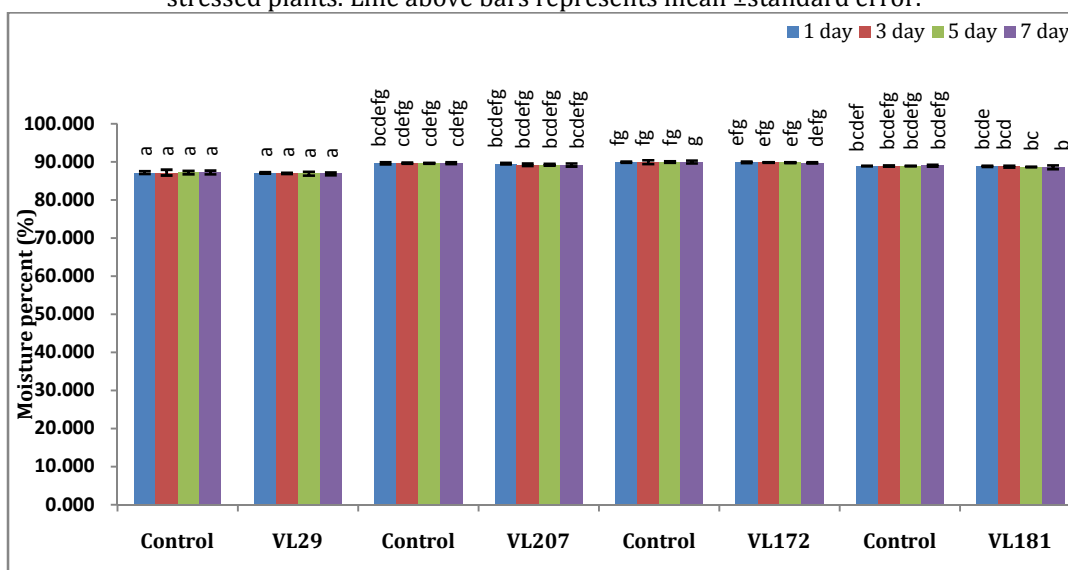


Fig 5 a

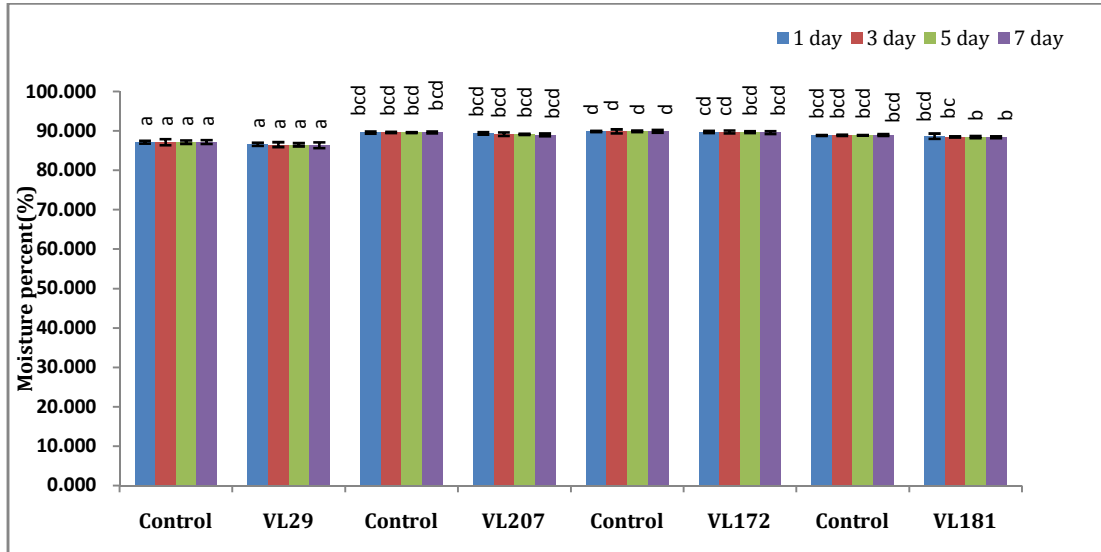


Fig 5 b

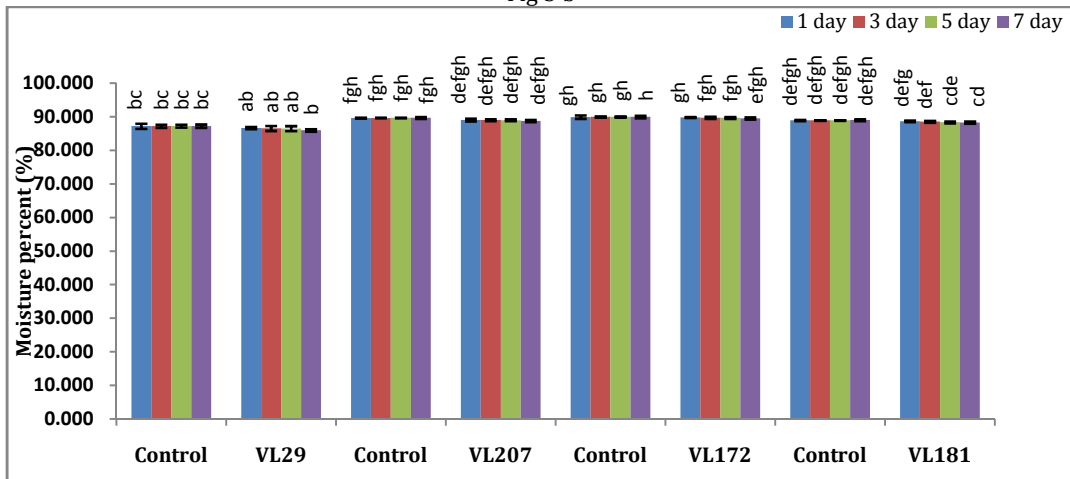


Fig 5 c

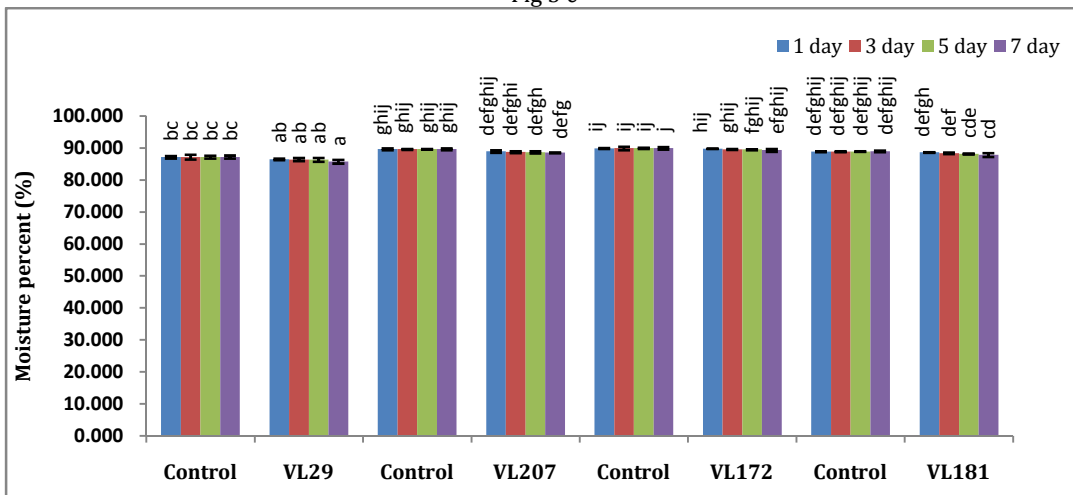


Fig 5 d

**Fig. 5.** Effect of progressive drought stress by different concentration of PEG (%) on moisture content in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences ( $P < 0.05$ ) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error

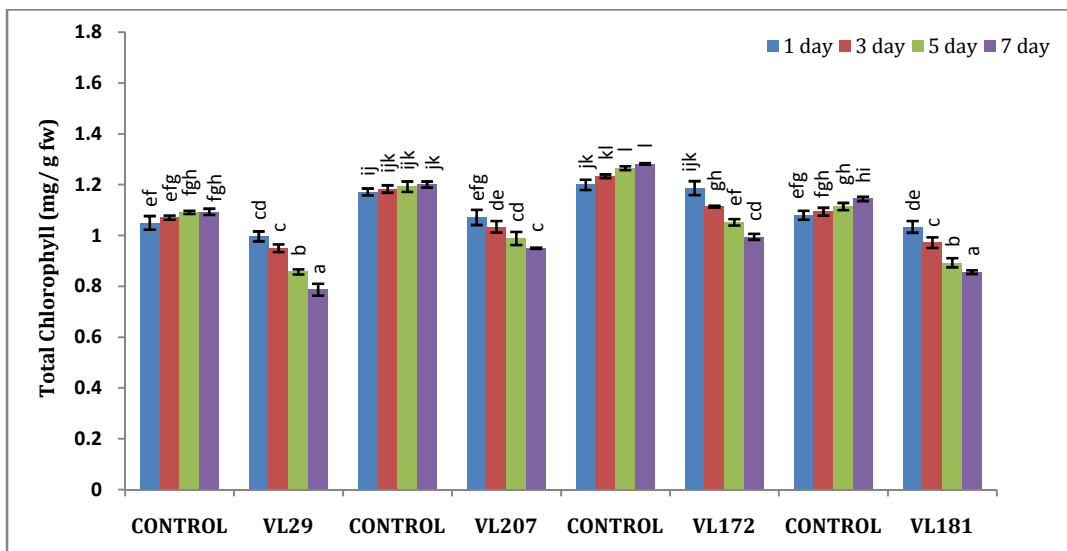


Fig 6 a

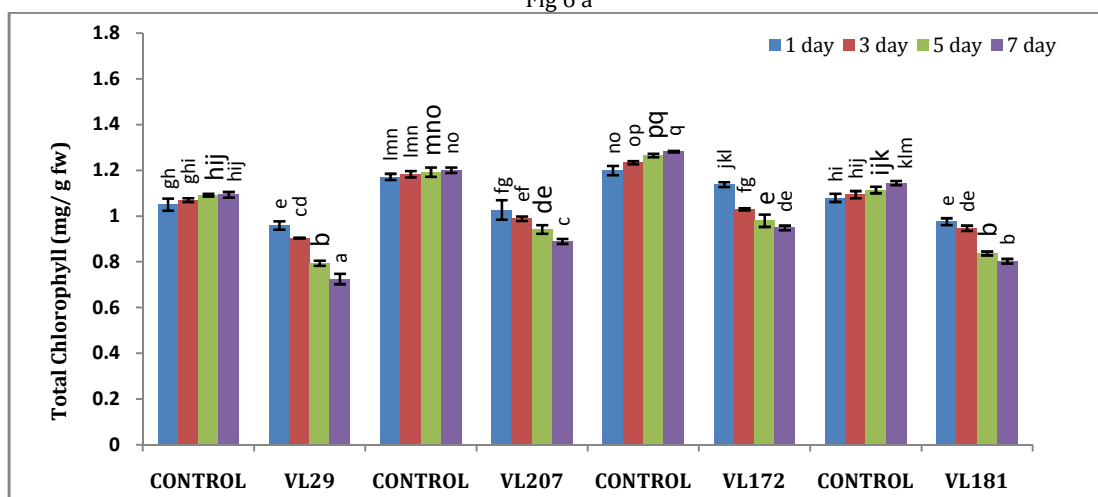


Fig 6 b

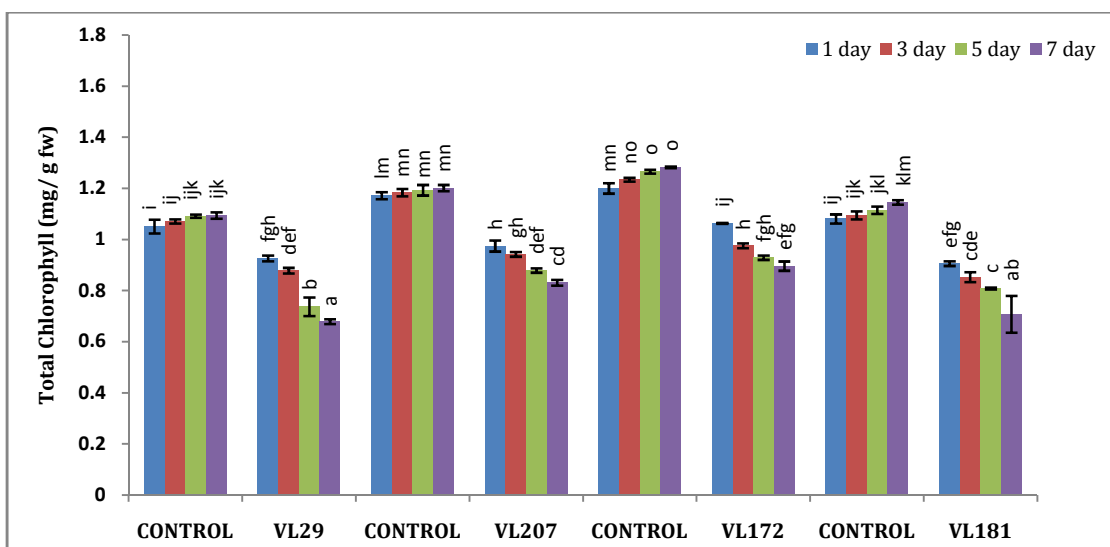


Fig 6 c

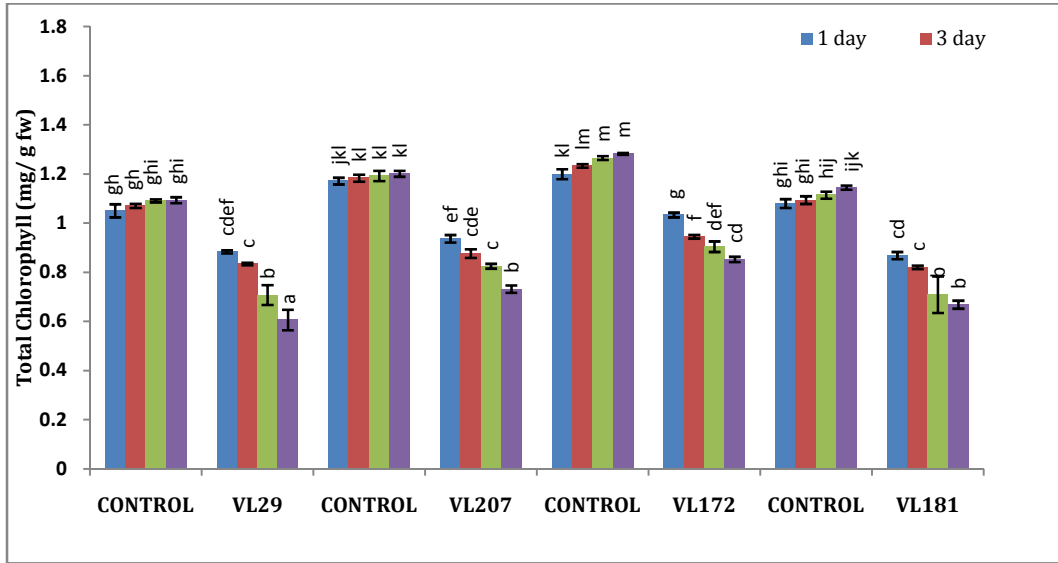


Fig 6 d

**Fig.6** Effect of progressive drought stress by different concentration of PEG (%) on Total chlorophyll content in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences (P<0.05) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error.

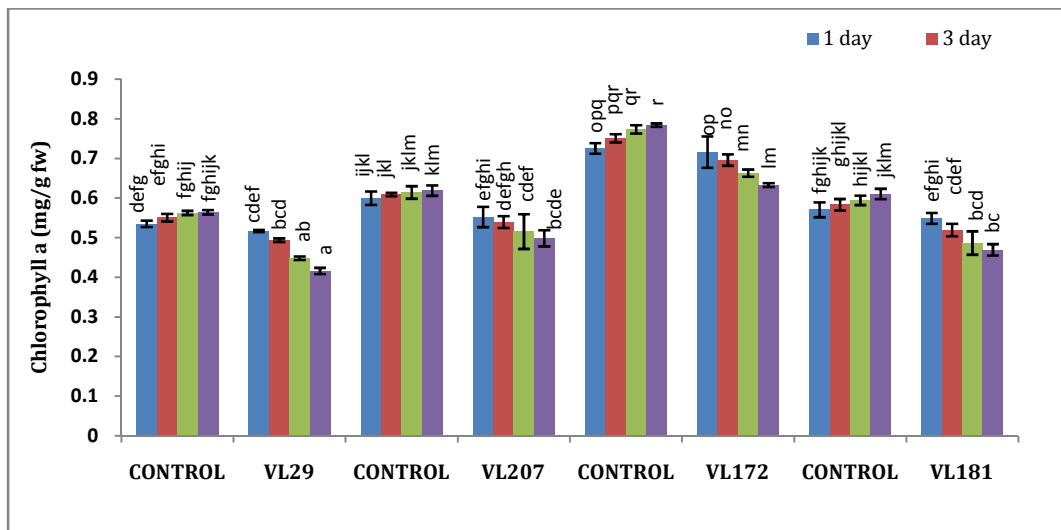


Fig 7 a

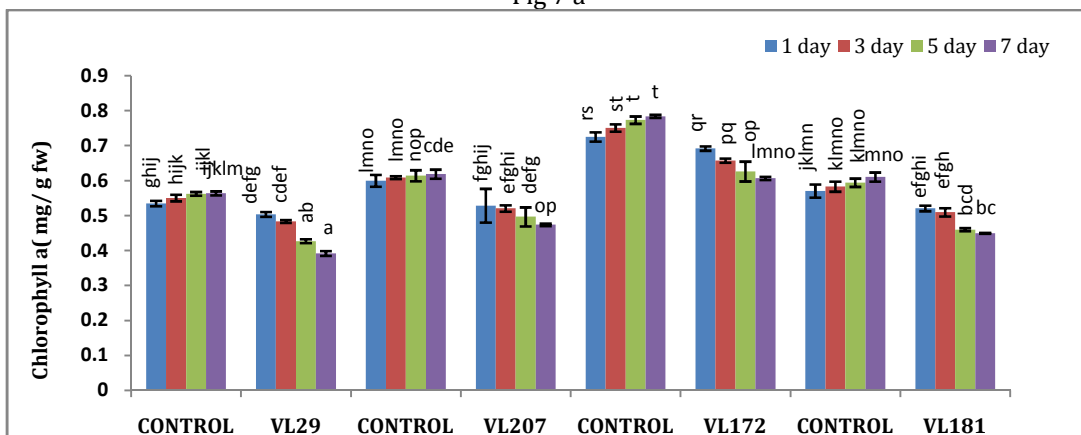


Fig 7 b

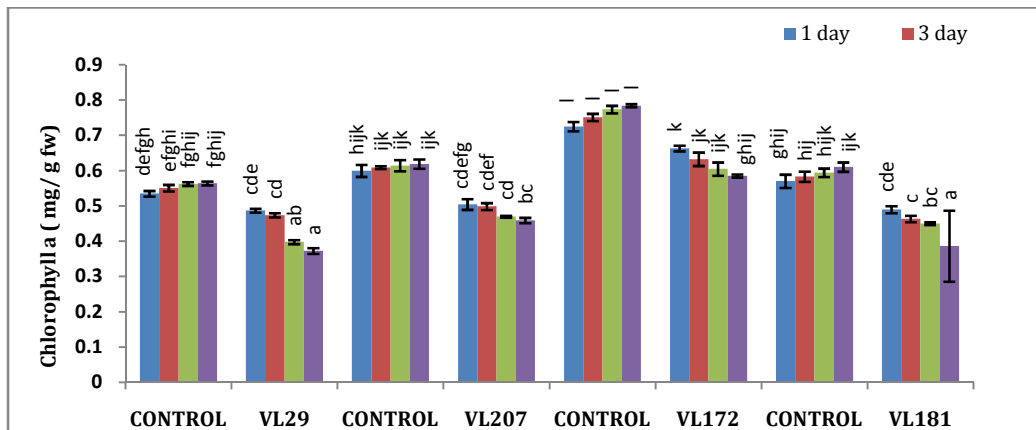


Fig 7 c

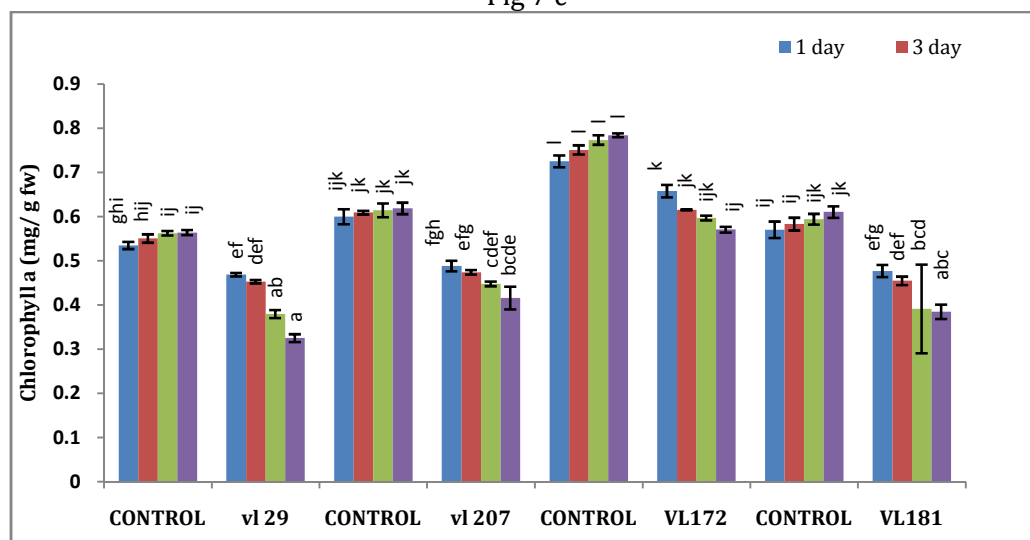


Fig 7d

**Fig.7** Effect of progressive drought stress by different concentration of PEG (%) on chlorophyll a in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences (P<0.05) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error.

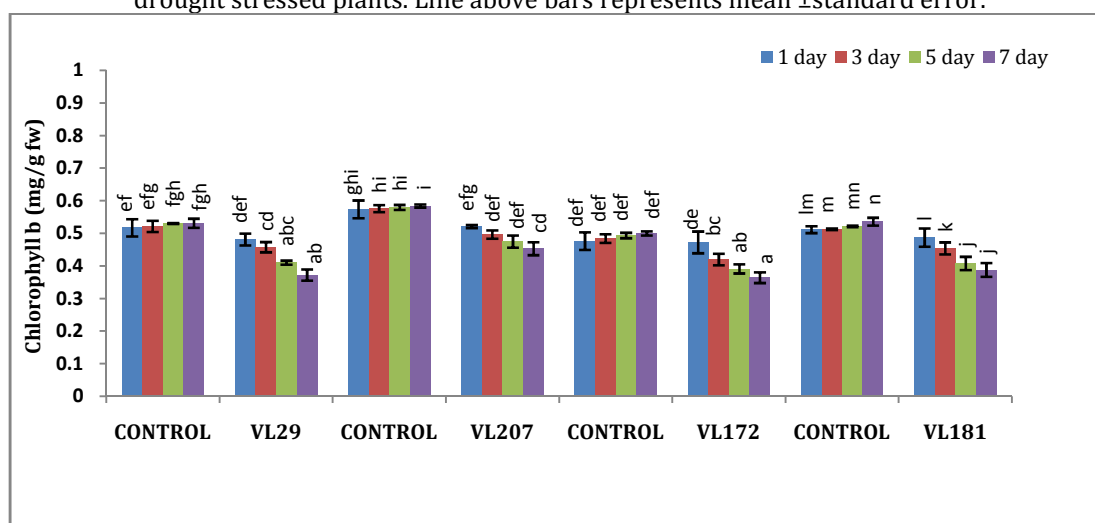


Fig 8a

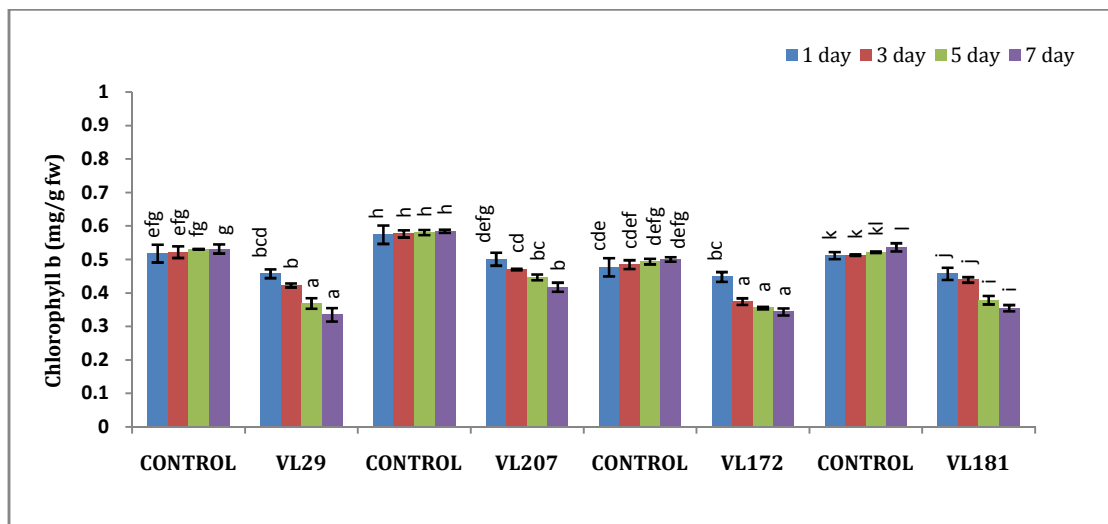


Fig 8b

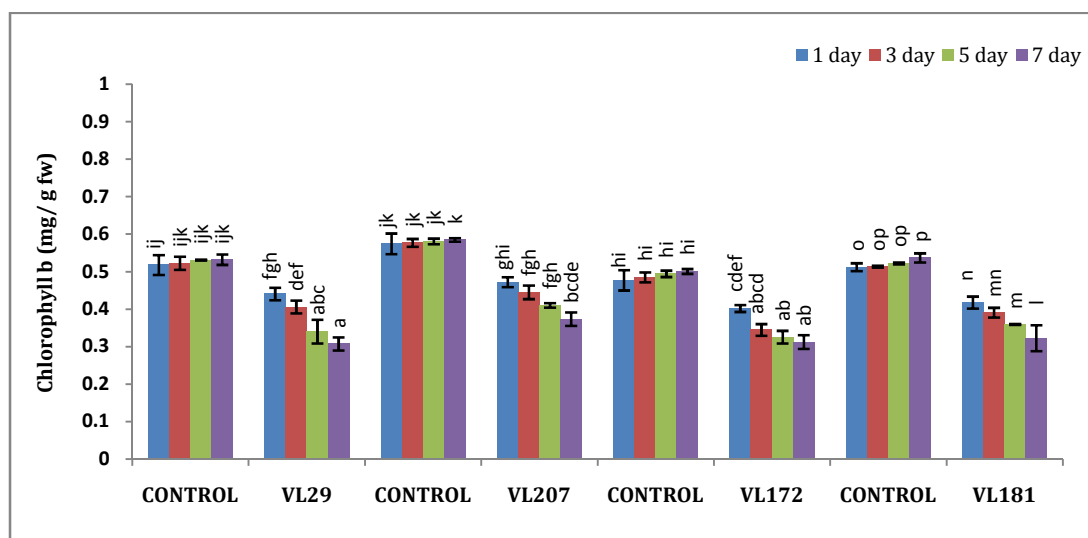


Fig 8c

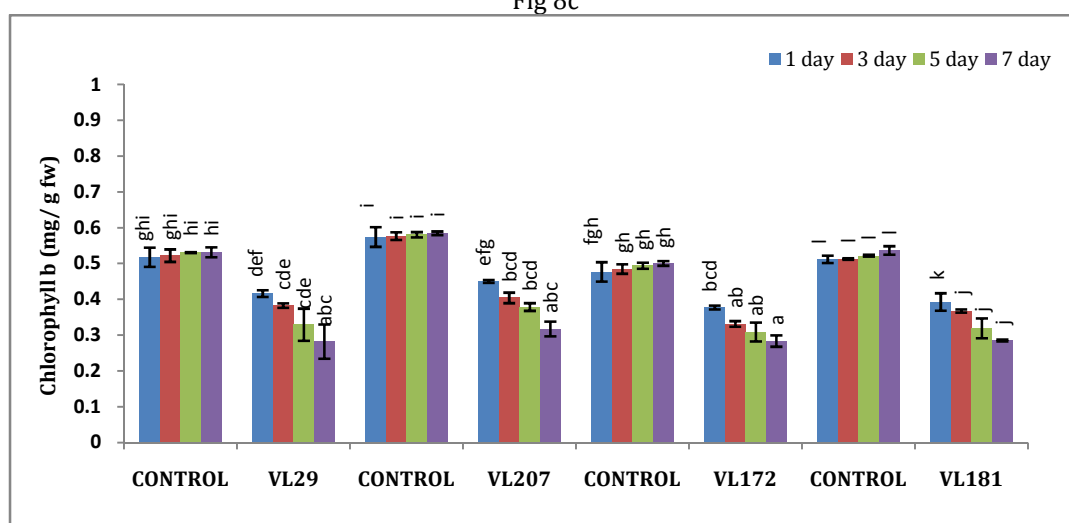


Fig 8d

**Fig. 8** Effect of progressive drought stress by different concentration of PEG (%) on chlorophyll bin four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences (P<0.05) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error.



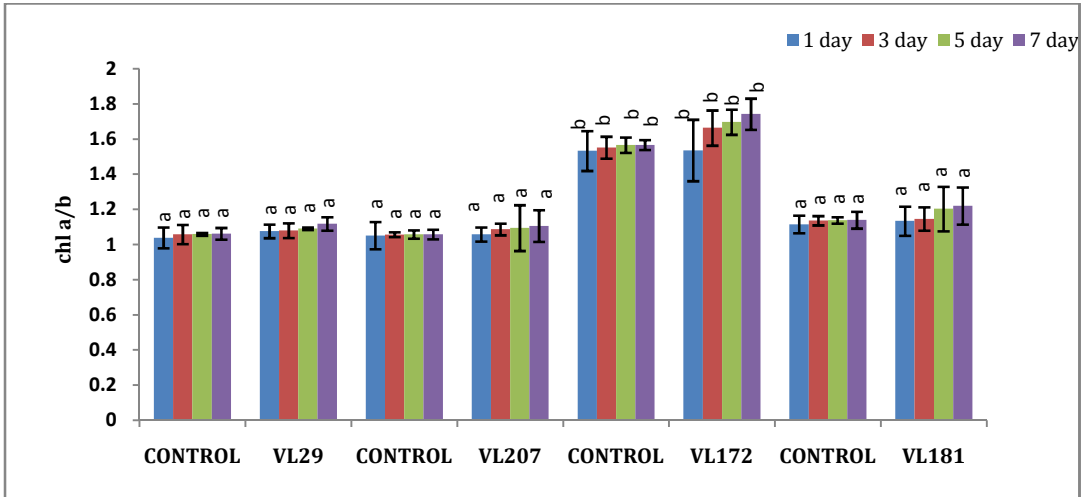


Fig 9a

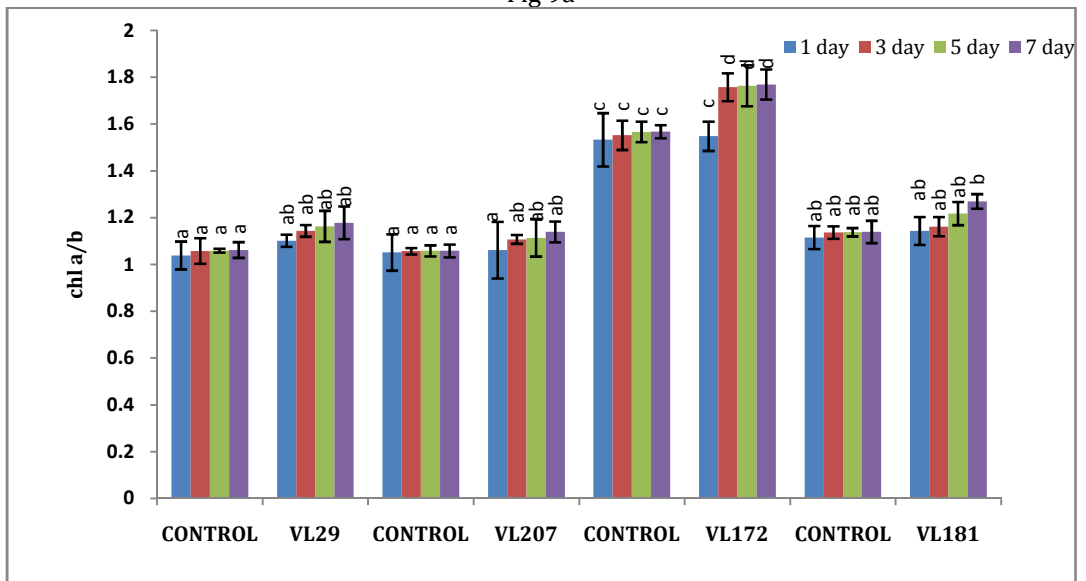


Fig 9b

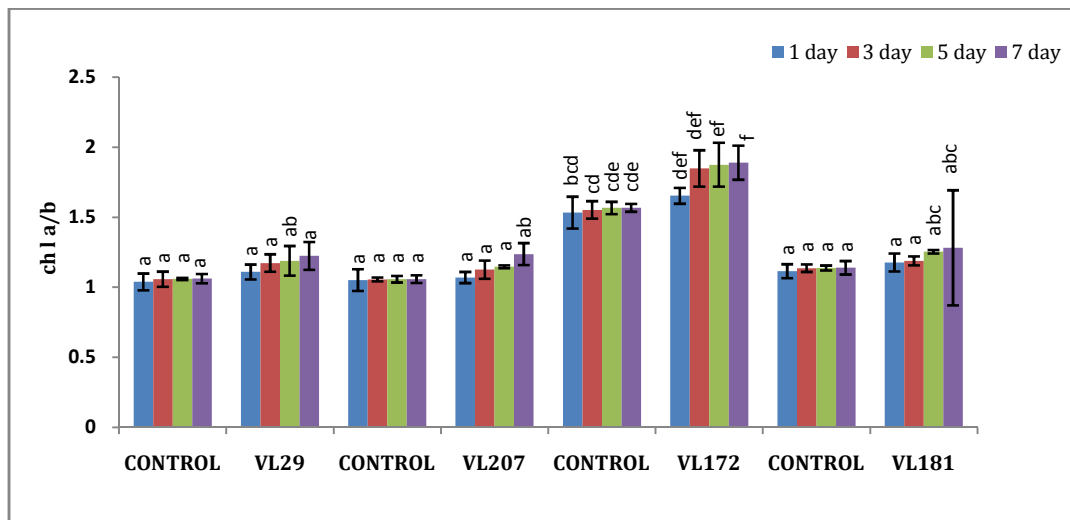


Fig 9c

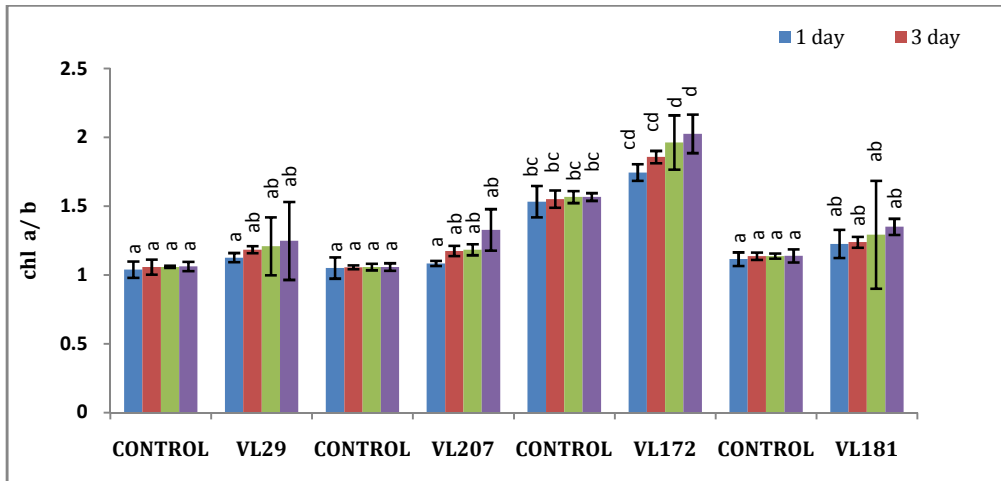


Fig 9d

**Fig.9** Effect of progressive drought stress by different concentration of PEG (%) on chlorophyll a/b in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences (P<0.05) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error.

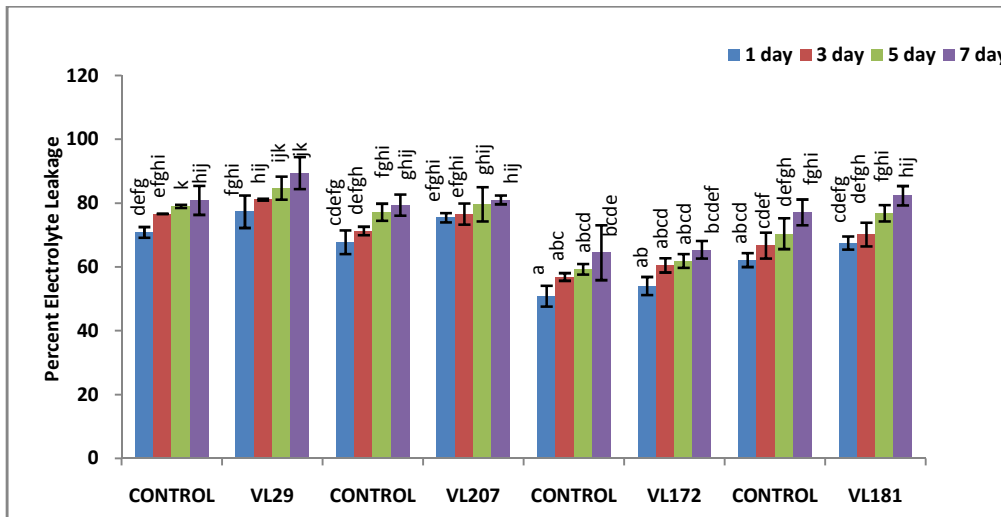


Fig 10a

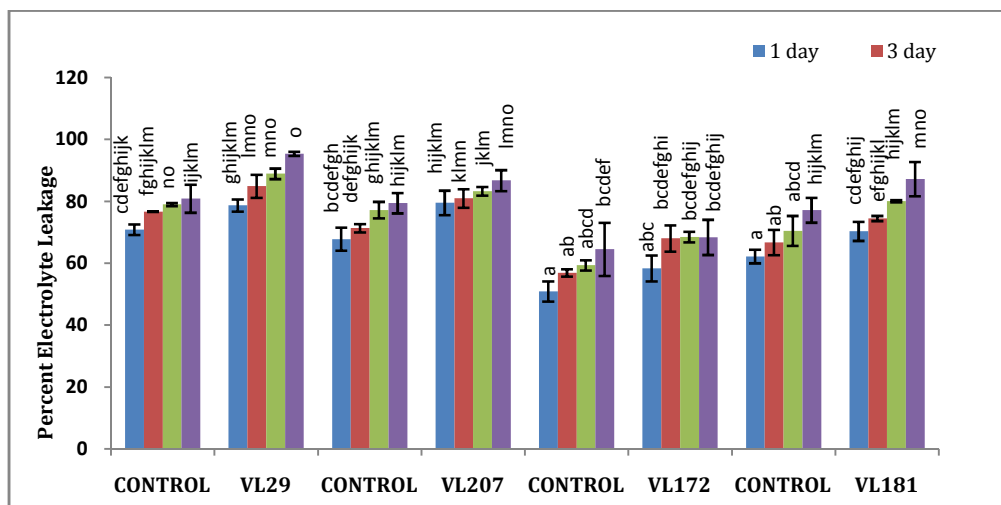


Fig 10b

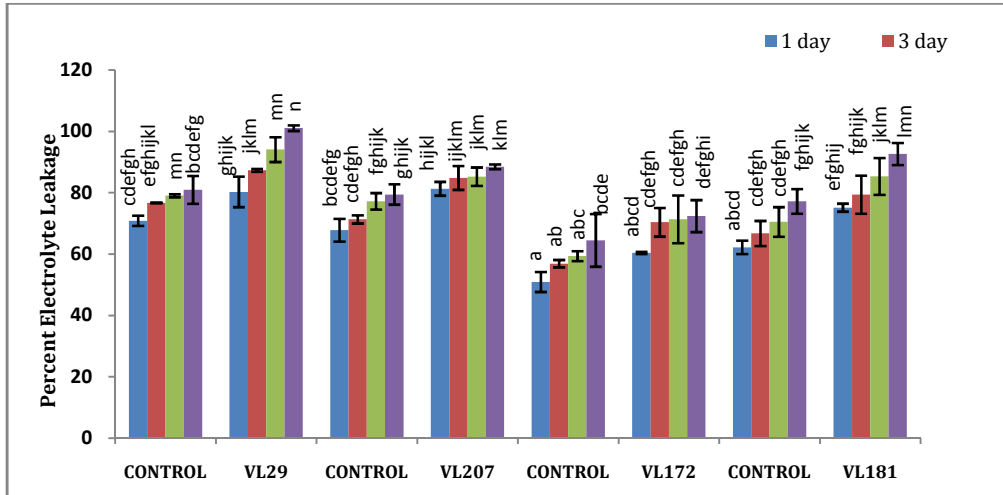


Fig 10c

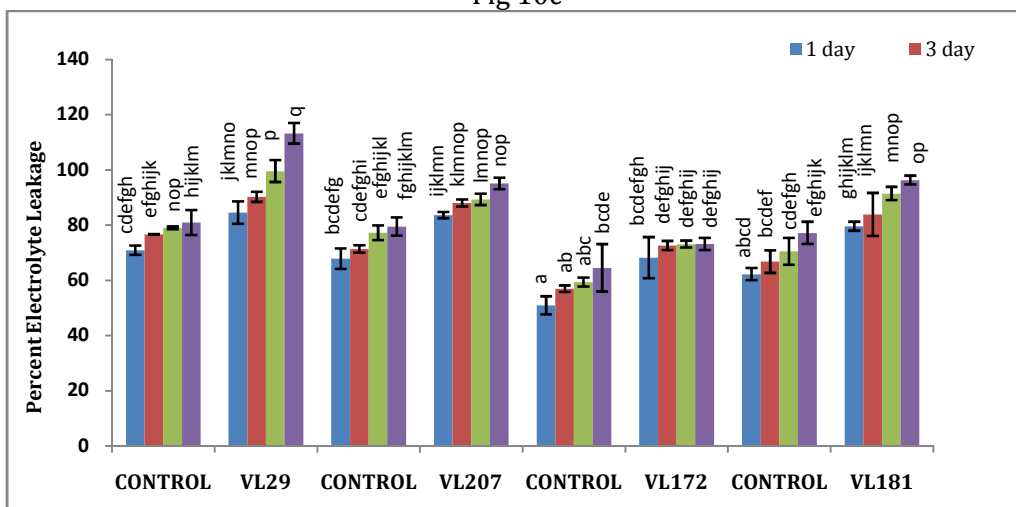
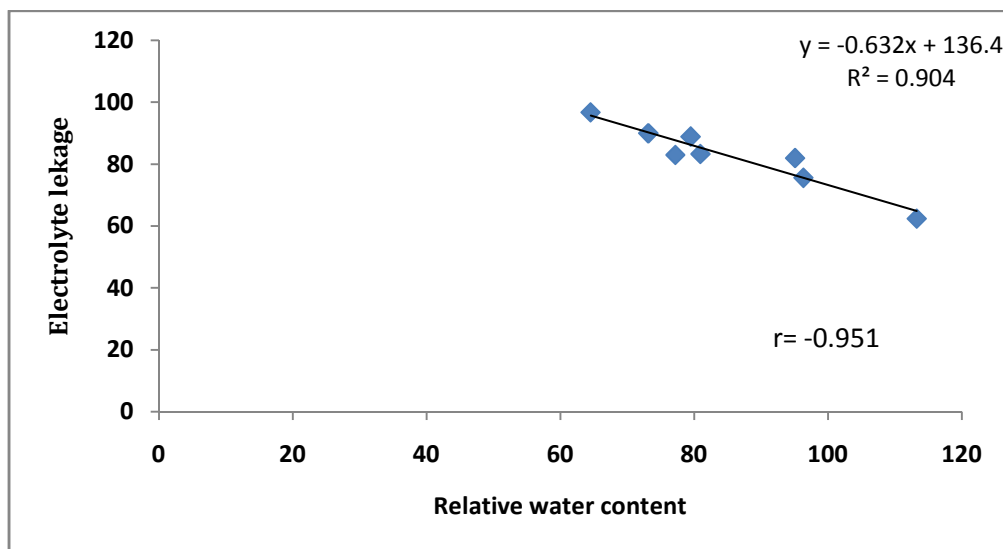


Fig 10d

**Fig.10** Effect of progressive drought stress by different concentration of PEG (%) on Electrolyte leakage in four varieties of barnyard millet (value represent mean  $\pm$  SE (n=3)). (a) 5% PEG (b) 10% PEG (c) 15% PEG (d) 20% PEG. Different letters denotes significant differences ( $P < 0.05$ ) among four varieties in control and drought stressed plants. Line above bars represents mean  $\pm$  standard error.



**Fig. 11** Correlation between relative water content and electrolyte leakage.

**CONCLUSION**

In the Himalayan region community is dependent on food as Barnyard millet which has very less productivity (8.63 quintal/ha) due to harsh climatic conditions. To improve the yield of barnyard millet, it is required to study the morphological and physiological response in presence of drought of the varieties grown in the Himalayan region. According to results of the present study variety VL172 found to be drought tolerant and variety VL29 drought susceptible among the others two varieties viz. VL207 and VL181. Variety VL172 can be further used for genomic study for exploring genes responsible on drought tolerant ability. That can further applicable for improvement of the plant by biotechnological approaches for superior variety.

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