Thesis of Doctoral (Ph.D.) Dissertation

Water Shortage Induced Response of Two Hungarian Potato (*Solanum tuberosum* L.) Genotypes

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1 SHORT INTRODUCTION AND OBJECTIVES

Potato (*Solanum tuberosum* L.) is the fifth most produced main crop in the world after sugar cane (*Saccharum officinarum* L.) wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.). Its production has increased from 314,208 thou-sand tonnes in 2007 to 388,191 thousand tonnes in 2017 (Statistics 2018). Modern cultivars are successful in improving tuber yield (Statistics 2018), yet they are sensitive to drought. Drought is multidimensional stress as it affects physiology, morphology, ecology, biochemical and molecular traits of plants (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Potato has shallow roots that make it prone to drought resulting from limited water availability (Watkinson et al. 2008). Several *in vitro* and field studies have been conducted to understand the effect of drought stress on potatoes (Schittenhelm et al. 2006; Hassanpanah 2010; Albiski et al. 2012; Stark et al. 2013). Reduction in the number of shoots (Albiski et al. 2012), plant height (Schittenhelm et al. 2006), leave numbers and area (Jefferies and MacKerron 1989), stolons (Haverkort et al. 1990), root length, and expansion (Albiski et al. 2012) have been reported in previous studies.

Plants have adopted various strategies to withstand drought stress through avoidance or tolerance (Yue et al. 2006). However, it is very complicated to characterize drought tolerance in potato cultivars as the different yields of different cultivars are not related to specific physiological or morphological traits (Stark et al. 2013). Different potato cultivars adapt to drought stress in different ways e.g., by higher assimilation portioning to tuber or by producing more tubers or by producing few but larger tubers (Deblonde and Ledent 2001). Understanding the mechanism of potato response to drought stress is a challenge to enhance crop drought tolerance. Water scarcity enforces the need for potato genotypes identification that exhibits high tolerance to drought stress (Anon. 2021). Widely used drought tolerance indicators in potatoes are leaf water content (Omae et al. 2005) and yield (Farshadfar and Elyasi 2012). Leaves are involved in photosynthesis and account for most of the water loss via transpiration; therefore, better canopy development, such as leaf shape, leaf areas, number of leaves, and stem length indicates a drought tolerance in potatoes (Schittenhelm et al. 2006). Under drought conditions leaf wilting is the most visual response to drought accompanied by a reduction in the number of leaves and stem length. Several agro-physiological parameters such as leaf area index, leaf area duration, chlorophyll content, and decrease in water loss have been established to be related to drought tolerance (Deblonde et al. 1999; Lahlou et al. 2003; Khan et al. 2015). Moreover, root system development has important implications for plant development and survival under drought conditions, as they absorb water and dissolved nutrients. Potatoes having a shallow root system; therefore, drought tolerance partially depends on root development as well (Joshi et al. 2016). Potato cultivars with larger and more expanded root systems are more likely to be able to retrieve water from the soil; therefore, being less susceptible to periodic drought (Wishart et al. 2013; Villordon et al. 2014). Measurement of the size and extent of the root system of different cultivars gives key information for breeding cultivars adapted to regions with frequent shortages of rainfall. That is why drought stress response of the genotypes can be observed by variation in several above ground and/or below ground plant development.

1.1 Objectives

This study aimed to describe the differences between two Hungarian bred potato genotypes' responses to drought in terms of the agro-physiological parameters and to establish which characters were the most related to the yield and/or drought tolerance.

The objectives are as follows:

a) Determine the effect of drought stress and identify any drought tolerance in Hungarian potato genotypes.

b) Determine the drought tolerance strategy of Hungarian potato genotypes.

c) Determine the variation and role of morphological characteristics of potato genotypes in drought tolerance.

d) Establish the relation between growth parameters and drought tolerance (if any) in potatoes.

e) Determining the plant developmental stage most susceptible to drought stress.

f) Determining the variation in chlorophyll content during different plant developmental stages and within plants.

2 MATERIALS AND METHODS

2.1 Plant Materials and Growth Conditions

To evaluate the effect of drought stress on potatoes a pot experiment was established in the greenhouse of Hungarian University of Agriculture and Life Sciences, Georgikon Campus Potato Research Centre, Keszthely, Hungary. For this purpose, soil and peat mixture (1:1 by weight) was used in 50 kg soil bearing pots (diameter at top = 41 cm; the height of pot = 40 cm) and a controlled environment (day/night temperature 25/21 °C, relative humidity 50%, and 18 h photoperiod) was maintained in the greenhouse. Two mid-late potato genotypes 'Demon' (G_1) and 'Hopehely' (G_2) were collected from Potato Research Centre, Keszthely, Hungary. Both genotypes are high-yielding and immune to potato Y and A virus, highly resistant to leaf drift virus. Both are moderately resistant to foliar phytophthora (*Phytophthora infestans*). Moreover, both are resistant to tuberculosis (*Mycobacterium tuberculosis*) and Ro1 and Ro4 potato nematode races.

2.2 Drought Stress Induction

Both genotypes were exposed to two water levels i.e., control (80% water holding capacity) and drought-stressed (50% water holding capacity). Drought stress was imposed at germination completion 18 days after sowing (DAS). Randomized complete block design (RCBD) was used with sixteen (16) replications. 5 kg pre-washed and dried gravel was used to line the pot base and was covered by a plastic net. A pipe was also embedded in the gravel for watering and aeration purposes. The remaining empty pot was filled with soil peat mixture (1:1 by weight). Soil from "A" horizon of a Eutric cambisol soil having a sandy clay loam texture was collected from the research farm area of the Hungarian University of Agriculture and Life Sciences, Georgikon Campus, and Baltic peat of low pH was brought from Latvia. Both soil and peat were sieved through a 10 mm sieve to obtain a finer and favourable growth medium. 10 kg of soil and 10 kg of peat were mixed using a cement mixer to obtain a homogenized mixture that was used as a growth medium in pots. The water holding capacity of the soil peat mixture was determined by the gravimetric method to quantify the amount of irrigation to be supplied to control and stressed pots. Pot weight was controlled weekly to ensure desired water level. 3 tubers per pot were sown. At germination completion (18 DAS) thinning was performed to maintain two plants per pot.

2.3 Sequential Harvesting

For growth analysis, 4 replications per treatment were harvested and sampled in 4 consecutive sampling times (S_1-S_4) during the experimental period on S_1 (36 DAS), S_2 (54 DAS), S_3 (72 DAS), and S_4 (90 DAS).

2.3.1 Morpho-physiological observations

Biomass was divided into leaves, stems, roots, and tubers. During 90 days experiment; data regarding plant height (cm), number of leaves per plant, foliage fresh weight (g/plant), foliage dry matter (g), numbers of tuber per plant, tubers weight (g/plant), root length (cm), and root fresh weight (g/plant) were recorded manually at each harvesting (S_1-S_4).

Leaf area index (LAI) was measured at each harvesting by using the formula described by Watson, (1947)

$$LAI = \frac{TOTAL\ LEAF\ AREA\ OF\ THE\ PLANT}{GROUND\ AREA\ OCCUPIED\ BY\ PLANT}$$

Leaf area duration (LAD) was measured at each harvesting by using the formula described by Power et al. (1967)

$$LAD = \frac{LAI_1 + LAI_2}{2} \times (t_2 - t_1)$$

where t_1 and t_2 are the time of first and second sampling and LAI_1 and LAI_2 are leaf area index at t_1 and t_2 respectively.

2.3.2 Relative Water Content

Relative water content (RWC) was measured at regular ten days intervals starting from first harvesting (36 DAS) up till senescence (76 DAS) by using the formula described by Barrs, (1968)

$$RWC = \frac{FRESH\ LEAF\ WEIGHT\ (FW) - DRY\ LEAF\ WEIGHT\ (DW)}{TURGID\ LEAF\ WEIGHT\ (TW) - DRY\ LEAF\ WEIGHT\ (DW)} \times 100$$

2.3.3 Chlorophyll content

Chlorophyll content in leaves was determined weekly by using SPAD-502 with the method described by Li et al. (2012) (Fig 1). SPAD values on the top leaflet, 1st side leaflets, and 2nd side leaflets of the 3rd, 4th, and 5th compound leaf from the apex, and at 3 points (top, middle and basal) within a leaflet were taken weekly after 45 DAS.

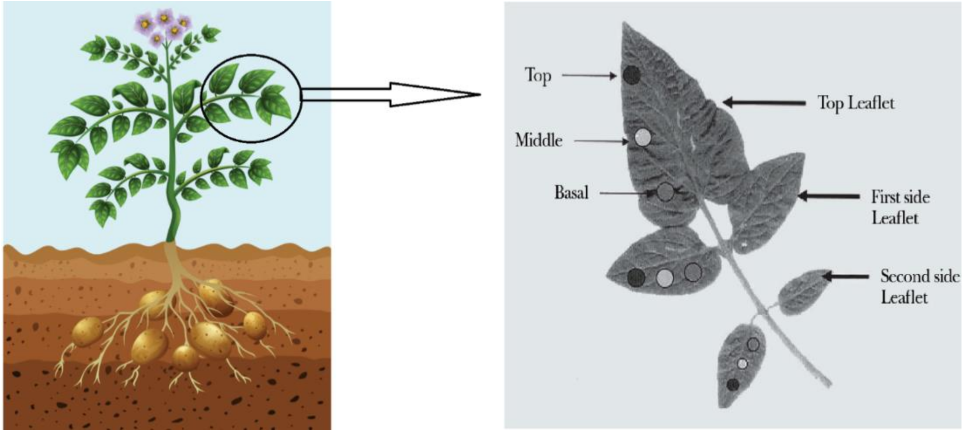


Figure 1 Diagram of a compound leaf, leaflet on the compound leaf, and measuring point on the leaflet of potato used for chlorophyll measurement (Li et al. 2012)

2.3.4 Nitrogen content in foliage

Nitrogen content (N%) in the foliage was also determined at each harvesting stage. To determine nitrogen content; foliage samples were sun-dried followed by oven drying. The dried samples were first ground using a Restch SN200 cutting mill and then further ground to dust-sized particles (10-50 µm) using Fritsch Analysette 3 Spartan Pulverisette 0. 100 mg ground samples were then placed in tin containers (8 mm × 55 mm) to deliver samples to Elementar Vario Macro Cube CHN analyzer (Germany) (Fig. 2) using 96 wells plates where N% was determined.

Figure 2 Elementar Vario Macro Cube CHNS analyzer (Germany)

2.3.5 Measurement of Enzymatic Antioxidant Activity

For enzymatic antioxidant activity, leaf samples were collected at 72 DAS and stored at -52 °C. Enzymes were extracted by adapting the published method by Yasmeen et al. (2014). To extract the enzymes, 0.5 g leaf samples were homogenized in 5 mL of 50 mM phosphate buffer with pH 7.8. The homogenate was then centrifuged at 15,000× *g* at 4 °C for 20 min. The supernatant was used to measure the superoxide dismutase (SOD) activity and catalase (CAT) activity. SOD and CAT activity was determined by following the procedure described by Giannopolitis and Ries (1977), and Chance and Maehly (1955), respectively.

2.4 Statistical Analysis

SPSS/PASW Statistics for Windows, version 18 (SPSS Inc., Chicago, USA) was used for statistical analysis. Experimental data were assessed for normality of distribution and homogeneity of variances. Analysis of variance (ANOVA) was performed to determine significant differences amongst treatments followed by Tukey's Honest Significant Difference test to recognize specific differences amongst treatments. Pearson correlation was performed to determine the relationship between variables. A $p<0.05$ was considered significant. Corrgrams were constructed using Statgraphics 19 centurion (STATGRAPHICS TECHNOLOGIES, Inc., Virginia, USA).

3 RESULTS AND DISCUSSION

3.1 Effect of drought stress on vegetative growth of potato genotypes

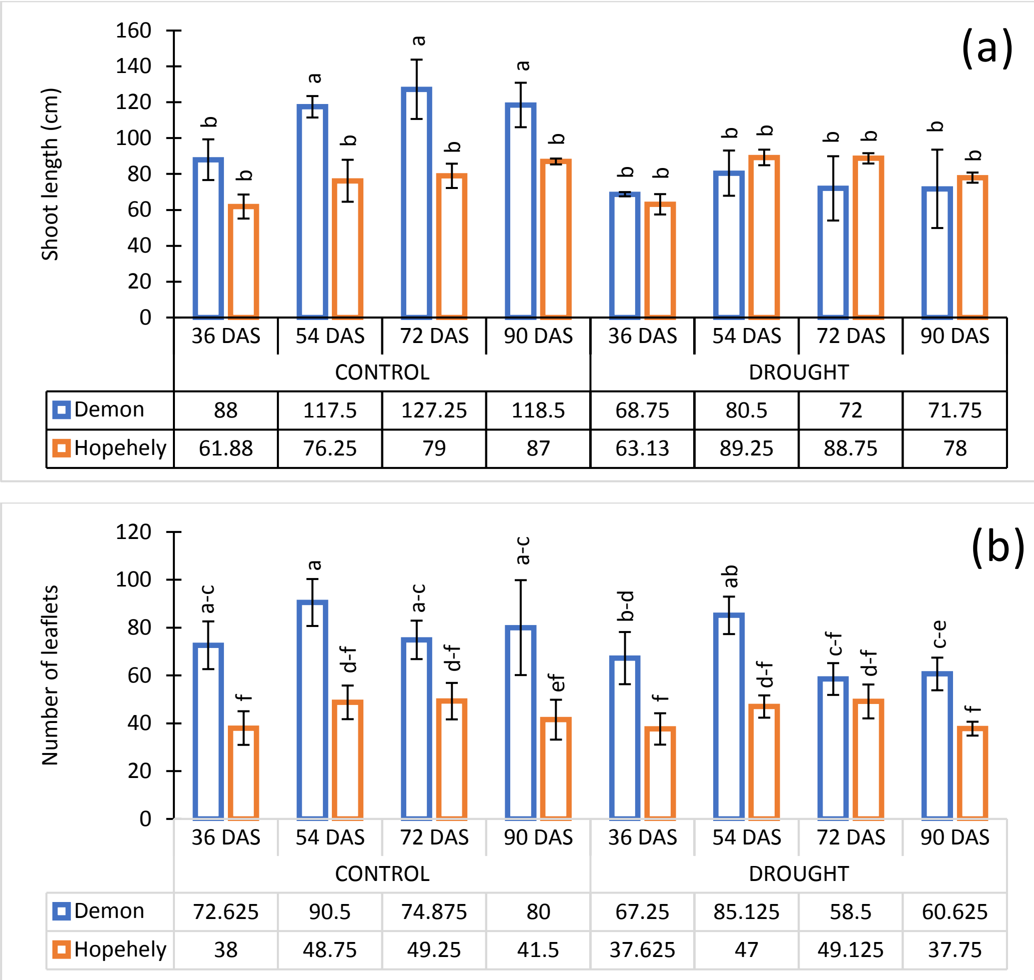
Analysis of variance (ANOVA) showed that genotype, drought stress, plant developmental stages and their interactions significantly affected ($p<0.05$) vegetative growth, underground growth and yield of studied potato genotypes. Vegetative growth of potatoes such as shoot length, number of leaflets, foliage dry weight and specific leaf weight (SLW) were significantly affected by genotype and plant development stage. Drought stress also showed significant effect on vegetative growth of potato plants except specific leaf weight ($p=0.47$) (Table 1).

Table 1 Analysis of variance for the effect of genotype, drought stress, plant developmental stages and their interaction on growth and yield of potato plants

Dependent variable	Source of variations (SOV)						
	A	B	C	A×B	A×C	B×C	A×B×C
Shoot length	0.00**	0.00**	0.00**	0.00**	0.96^{ns}	0.06^{ns}	0.04*
No. of leaflets	0.00**	0.00**	0.00**	0.03*	0.01**	0.46^{ns}	0.62^{ns}
Specific leaf weight	0.00**	0.47^{ns}	0.00**	0.00**	0.29^{ns}	0.62^{ns}	0.32^{ns}
Foliage dry weight	0.00**	0.01**	0.00**	0.01**	0.06^{ns}	0.00**	0.02*
Leaf area index	0.10^{ns}	0.01**	0.00**	0.00**	0.35^{ns}	0.19^{ns}	0.06^{ns}
Leaf area duration	0.05*	0.01**	0.00**	0.00**	0.24^{ns}	0.20^{ns}	0.17^{ns}
Chlorophyll content	0.00**	0.07^{ns}	0.00**	0.00**	0.00**	0.00**	0.00**
Nitrogen content	0.00**	0.12^{ns}	0.00**	0.01**	0.32^{ns}	0.40^{ns}	0.44^{ns}
Relative water content	0.00**	0.64^{ns}	0.00**	0.02*	0.02*	0.28^{ns}	0.00**
Fresh plant biomass	0.84^{ns}	0.00**	0.00**	0.00**	0.02*	0.00**	0.34^{ns}
Root length	0.29^{ns}	0.98^{ns}	0.75^{ns}	0.82^{ns}	0.05^{ns}	0.53^{ns}	0.95^{ns}
Root dry weight	0.01**	0.04*	0.00**	0.11^{ns}	0.00**	0.04*	0.28^{ns}
No. of tubers	0.00**	0.01**	0.00**	0.01**	0.00**	0.14^{ns}	0.07^{ns}
Fresh tuber yield	0.00**	0.30^{ns}	0.00**	0.00**	0.00**	0.02*	0.15^{ns}

Where, A = genotypes, B = water levels, C = plant developmental stage, $^{*}p<0.05$, $^{*}p<0.01$, $^{ns}p>0.05$

Under control conditions 'Demon' produced significantly taller plants than 'Hopehely' but drought stress significantly reduced plant height of 'Demon'. However, 'Hopehely' maintained its plant height under drought stress conditions. 'Demon' produced significantly higher number of leaflets than 'Hopehely' under control as well as drought conditions. But drought stress showed a significant effect on number of leaflets of 'Demon' while 'Hopehely' maintained its number of leaflets under stress conditions (Fig 3).



(a)

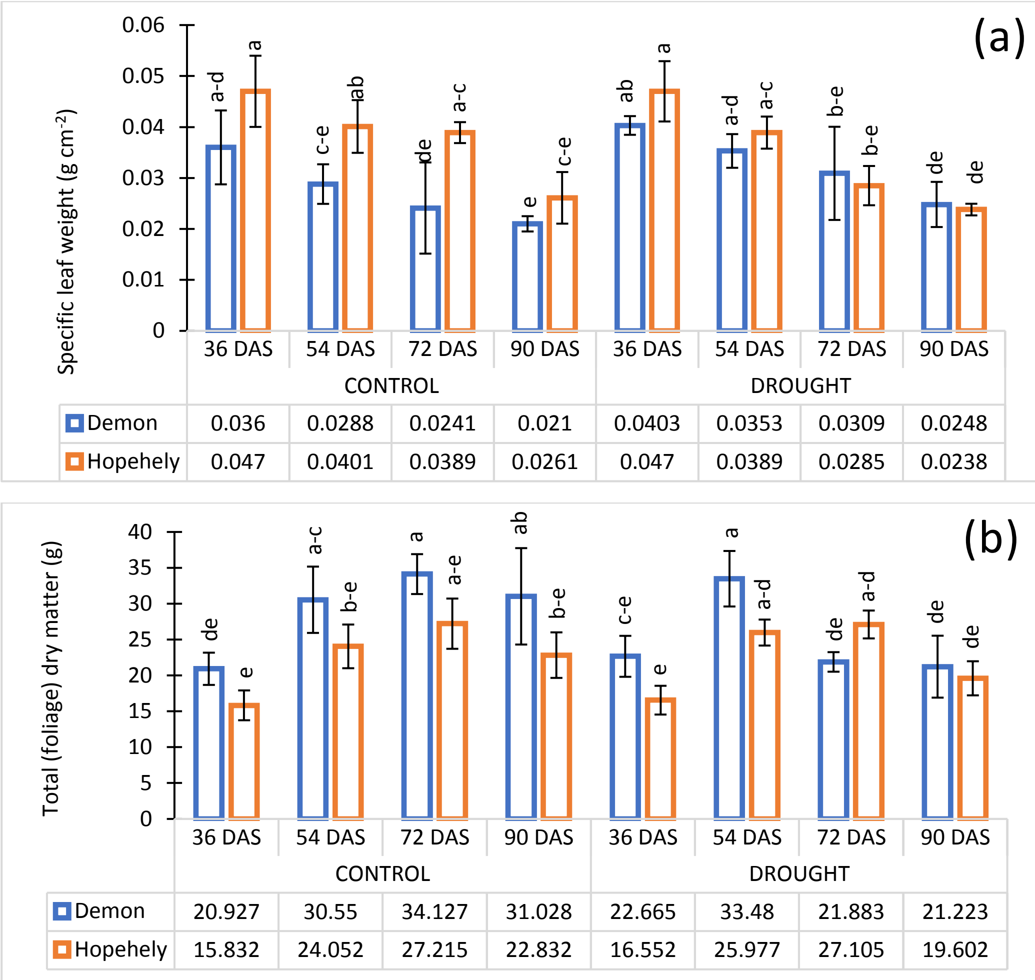
	CONTROL 36 DAS	CONTROL 54 DAS	CONTROL 72 DAS	CONTROL 90 DAS	DROUGHT 36 DAS	DROUGHT 54 DAS	DROUGHT 72 DAS	DROUGHT 90 DAS
Demon	88	117.5	127.25	118.5	68.75	80.5	72	71.75
Hopehely	61.88	76.25	79	87	63.13	89.25	88.75	78

(b)

	CONTROL 36 DAS	CONTROL 54 DAS	CONTROL 72 DAS	CONTROL 90 DAS	DROUGHT 36 DAS	DROUGHT 54 DAS	DROUGHT 72 DAS	DROUGHT 90 DAS
Demon	72.625	90.5	74.875	80	67.25	85.125	58.5	60.625
Hopehely	38	48.75	49.25	41.5	37.625	47	49.125	37.75

Figure 3 Effect of drought stress on: (a) shoot length; (b) number of leaflets of potato genotypes. Each value is mean ± STDEV (standard deviation) of four replicates. Different letters indicate groups were significantly different ($p < 5\%$) according to Tukey's post-hoc test.

'Hopehely' produced significantly higher SLW than 'Demon' under control as well as drought condition. Both genotypes reached maximum SLW at early plant developmental stage that significantly decreased at further plant developmental stages. Under control conditions, both genotypes reached peak foliage dry mass production at tuber bulking stage that significantly decreased afterwards. However, under drought stress, 'Demon' plants reached maximum foliage dry mass production at flowering stage that significantly decreased afterwards (Fig 4).



	CONTROL 36 DAS	CONTROL 54 DAS	CONTROL 72 DAS	CONTROL 90 DAS	DROUGHT 36 DAS	DROUGHT 54 DAS	DROUGHT 72 DAS	DROUGHT 90 DAS
Demon	0.036	0.0288	0.0241	0.021	0.0403	0.0353	0.0309	0.0248
Hopehely	0.047	0.0401	0.0389	0.0261	0.047	0.0389	0.0285	0.0238

	CONTROL 36 DAS	CONTROL 54 DAS	CONTROL 72 DAS	CONTROL 90 DAS	DROUGHT 36 DAS	DROUGHT 54 DAS	DROUGHT 72 DAS	DROUGHT 90 DAS
Demon	20.927	30.55	34.127	31.028	22.665	33.48	21.883	21.223
Hopehely	15.832	24.052	27.215	22.832	16.552	25.977	27.105	19.602

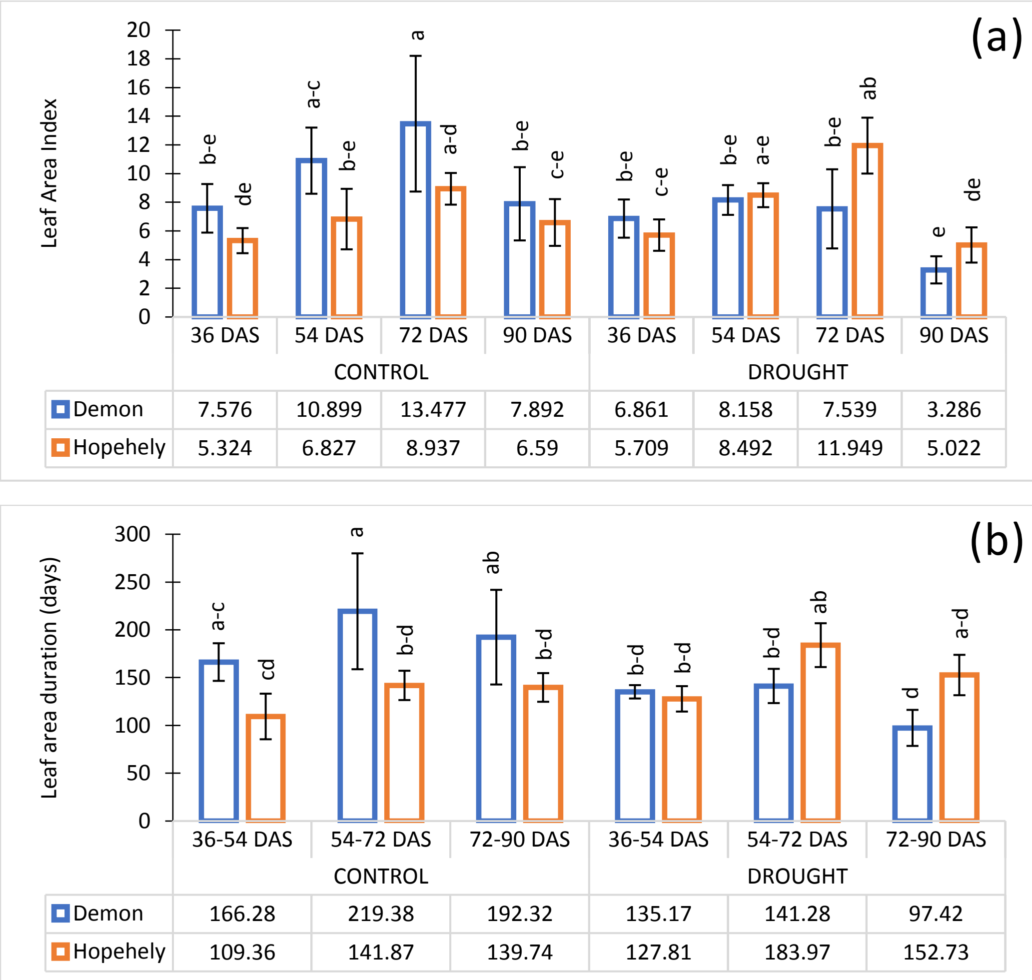
Figure 4 Effect of drought stress on: (a) specific leaf weight; (b) foliage dry matter of potato genotypes. Each value is mean ± STDEV (standard deviation) of four replicates. Different letters indicate groups were significantly different ($p < 5\%$) according to Tukey's post-hoc test.

3.2 Effect of drought stress on LAI and LAD of potato genotypes

Factorial ANOVA showed that leaf area index (LAI) ($f(1,45)=18.37$, $p<0.01$) and leaf area duration (LAD) ($f(1,33)=33.95$, $p<0.01$) were significantly affected by genotype and drought stress interaction. Simple main effects showed that genotype have statistically significant effect ($p=0.05$) on LAD but drought stress ($p=0.01$) and plant developmental stages ($p<0.01$) had a significant effect on both, LAI and LAD (Table 1).

Highest LAI under control conditions was calculated for ‘Demon’ plants (13.47) while under drought stress for ‘Hopehely’ plants (11.94). Tukey post-hoc test revealed that drought stress significantly reduced LAI of ‘Demon’. However, non-significant increase in LAI was observed for ‘Hopehely’ plants under drought stress conditions. LAI of both genotypes increased till tuber bulking stage and then significant reduction was observed between tuber bulking and senescence (Fig. 5).

Under control conditions, LAD of ‘Demon’ was significantly higher than ‘Demon’ but drought stress significantly reduced LAD of ‘Demon’. On the other hand, LAD of ‘Hopehely’ was higher under drought stress but statistically at par with control conditions. LAD of both genotypes was highest during flowering and tuber bulking stage (54-72 DAS). ‘Demon’ plants under drought stress reached the senescence earliest while other plants still had green leaves on them (Fig. 5).



	CONTROL 36 DAS	CONTROL 54 DAS	CONTROL 72 DAS	CONTROL 90 DAS	DROUGHT 36 DAS	DROUGHT 54 DAS	DROUGHT 72 DAS	DROUGHT 90 DAS
Demon	7.576	10.899	13.477	7.892	6.861	8.158	7.539	3.286
Hopehely	5.324	6.827	8.937	6.59	5.709	8.492	11.949	5.022

	CONTROL 36-54 DAS	CONTROL 54-72 DAS	CONTROL 72-90 DAS	DROUGHT 36-54 DAS	DROUGHT 54-72 DAS	DROUGHT 72-90 DAS
Demon	166.28	219.38	192.32	135.17	141.28	97.42
Hopehely	109.36	141.87	139.74	127.81	183.97	152.73

Figure 5 Effect of drought stress on: (a) leaf area index; (b) leaf area duration of potato genotypes. Each value is mean ± STDEV (standard deviation) of four replicates. Different letters indicate groups were significantly different (p < 5%) according to Tukey's post-hoc test.

3.3 Effect of drought stress on chlorophyll content, nitrogen content and relative water content of potato genotypes

Statistical analysis showed that genotype and plant developmental stages had a significant effect ($p<0.01$) on plant chlorophyll content, nitrogen content in foliage and relative water content (RWC) of leaves. Factorial analysis also revealed that all possible interactions between the effects of independent variables on chlorophyll content were statistically significant ($p<0.01$). A statistically significant interaction between the effect of genotype and drought stress ($f(1,45)$-7.39, $p=0.01$) was observed on nitrogen content in the foliage, while there was no statistically significant interaction between any other independent variables. Drought stress did not show any significant effect ($p=0.64$) on RWC but its interaction with genotype had a statistically significant effect ($f(1,45)=6.28$, $p=0.02$) on RWC (Table 1).

Under drought stress significantly higher nitrogen content was observed in ‘Hopehely’ than ‘Demon’. Non-significant decrease in nitrogen content was observed in ‘Demon’ due to drought stress while in ‘Hopehely’ non-significant increase was observed in response to drought stress. Under control and stress conditions, both genotypes produced the highest nitrogen content at tuber initiation stage. Nitrogen content in foliage of both genotypes significantly decreased at each plant development stage. Lowest nitrogen content (1.11%) was observed in ‘Demon’ plants at senescence under drought stress conditions. Water content in the leaves of ‘Hopehely’ was significantly higher than ‘Demon’. Under control conditions, RWC of both genotypes was statistically at par at each developmental stage. Under drought stress, RWC of ‘Hopehely’ was significantly higher than ‘Demon’ at each developmental stage except at the initiation of flowering (46 DAS). Moreover, drought stress significantly reduced RWC of ‘Demon’ at flowering and senescence stage (Fig. 6).

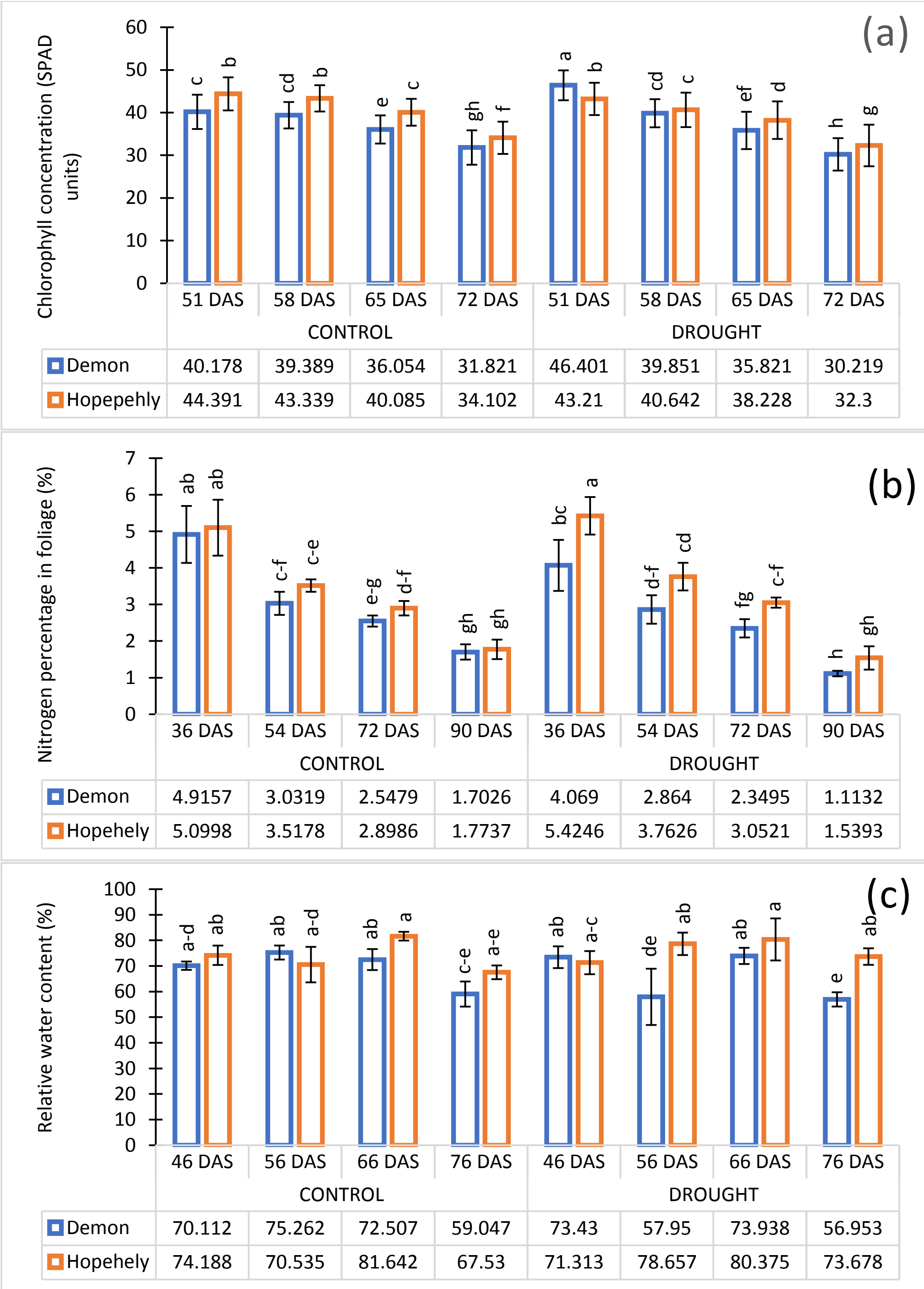
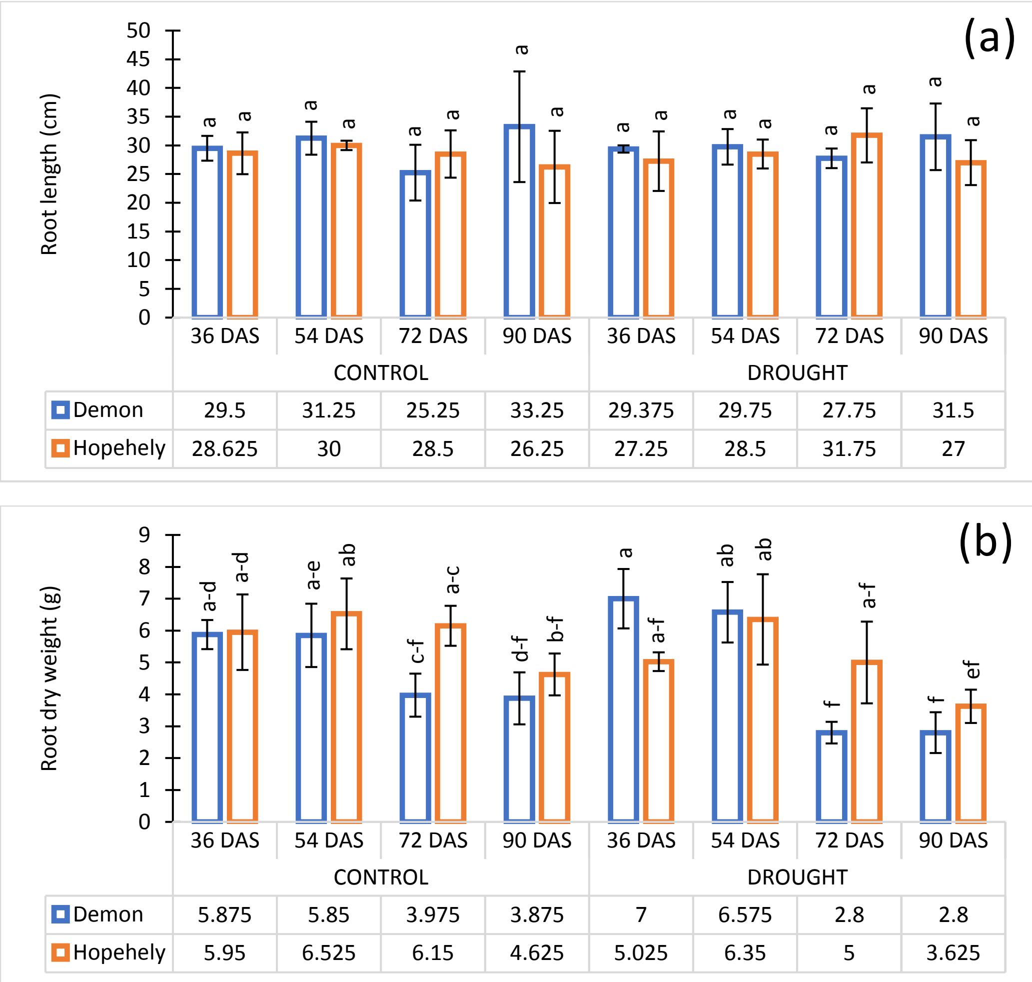


Figure 6 Effect of drought stress on: (a) chlorophyll content; (b) nitrogen content; (c) relative water content of potato genotypes. Each value is mean ± STDEV (standard deviation) of four replicates. Different letters indicate groups were significantly different (p < 5%) according to Tukey's post-hoc test.

3.4 Effect of drought stress on roots of potato genotypes

Analysis of variance showed that root length was not affected by either of the independent variables or their interaction ($p>0.05$), while genotype ($p=0.01$), drought stress ($p=0.04$) and plant developmental stages ($p<0.01$) had a statistically significant effect on root dry weight. Genotype×drought stress also showed statistically significant effect ($f(3,45=8.89$, $p<0.01$) on root dry weight. Factorial ANOVA also revealed a statistically significant interaction between the effects of drought stress and plant developmental stage ($f(3,45=2.93$, $p=0.04$) (Table 1).



(a) Root length (cm)

	CONTROL 36 DAS	CONTROL 54 DAS	CONTROL 72 DAS	CONTROL 90 DAS	DROUGHT 36 DAS	DROUGHT 54 DAS	DROUGHT 72 DAS	DROUGHT 90 DAS
Demon	29.5	31.25	25.25	33.25	29.375	29.75	27.75	31.5
Hopehely	28.625	30	28.5	26.25	27.25	28.5	31.75	27

(b) Root dry weight (g)

	CONTROL 36 DAS	CONTROL 54 DAS	CONTROL 72 DAS	CONTROL 90 DAS	DROUGHT 36 DAS	DROUGHT 54 DAS	DROUGHT 72 DAS	DROUGHT 90 DAS
Demon	5.875	5.85	3.975	3.875	7	6.575	2.8	2.8
Hopehely	5.95	6.525	6.15	4.625	5.025	6.35	5	3.625

Figure 7 Effect of drought stress on (a) root length; (b) root dry weight of potato genotypes. Each value is mean ± STDEV (standard deviation) of four replicates. Different letters indicate groups were significantly different ($p < 5\%$) according to Tukey's post-hoc test.

‘Demon’ plants reached maximum root dry weight earlier (36 DAS) compared to ‘Hopehely’ that reached maximum root dry weight at flowering (54 DAS) under control and drought stress. Highest root dry weight was recorded for ‘Demon’ plants under drought stress at tuber initiation stage that was statistically at par with root dry weight of ‘Hopehely’ at tuber initiation and bulking stage under control and drought stress conditions. Drought stress significantly reduced root dry weight of ‘Demon’ during flowering and tuber bulking stage (Fig. 7).

3.5 Effect of drought stress on yield of potato genotypes

We observed a statistically significant effect of genotypes ($p<0.01$), drought stress ($p<0.01$), plant developmental stage ($p<0.01$), genotype$\times$drought stress ($f(1,45)=6.45$, $p=0.01$), and drought stress$\times$plant developmental stage ($f(3,45)=6.27$, $p<0.01$) on the number of tubers produced by plants. Tuber yield was significantly affected by genotype ($p<0.01$) and plant developmental stages ($p<0.03$) but drought stress did not show any significant effect on tuber yield ($p=0.30$). Factorial analysis revealed that there was a statistically significant interaction between the effects of genotype and drought stress ($f(1,45)=11.84$, $p<0.01$), genotype and plant developmental stages ($f(3,45)=9.82$, $p<0.01$) and drought stress and plant developmental stage ($f(3,45)=3.54$, $p=0.02$) (Table 1).

‘Hopehely’ produced significantly higher tuber yield than ‘Demon’ under control and drought. Under both growing conditions, tuber yield of both genotypes increased significantly at each developmental stage reaching maximum at 90 DAS. Highest tuber yield was observed for ‘Hopehely’ under control conditions (664.8 g) that was statistically at par with its yield under drought stress (598.2 g). A significant increase in ‘Demon’ tuber yield was observed at flowering stage under drought stress compared to its tuber yield under well-watered conditions (Fig. 8).

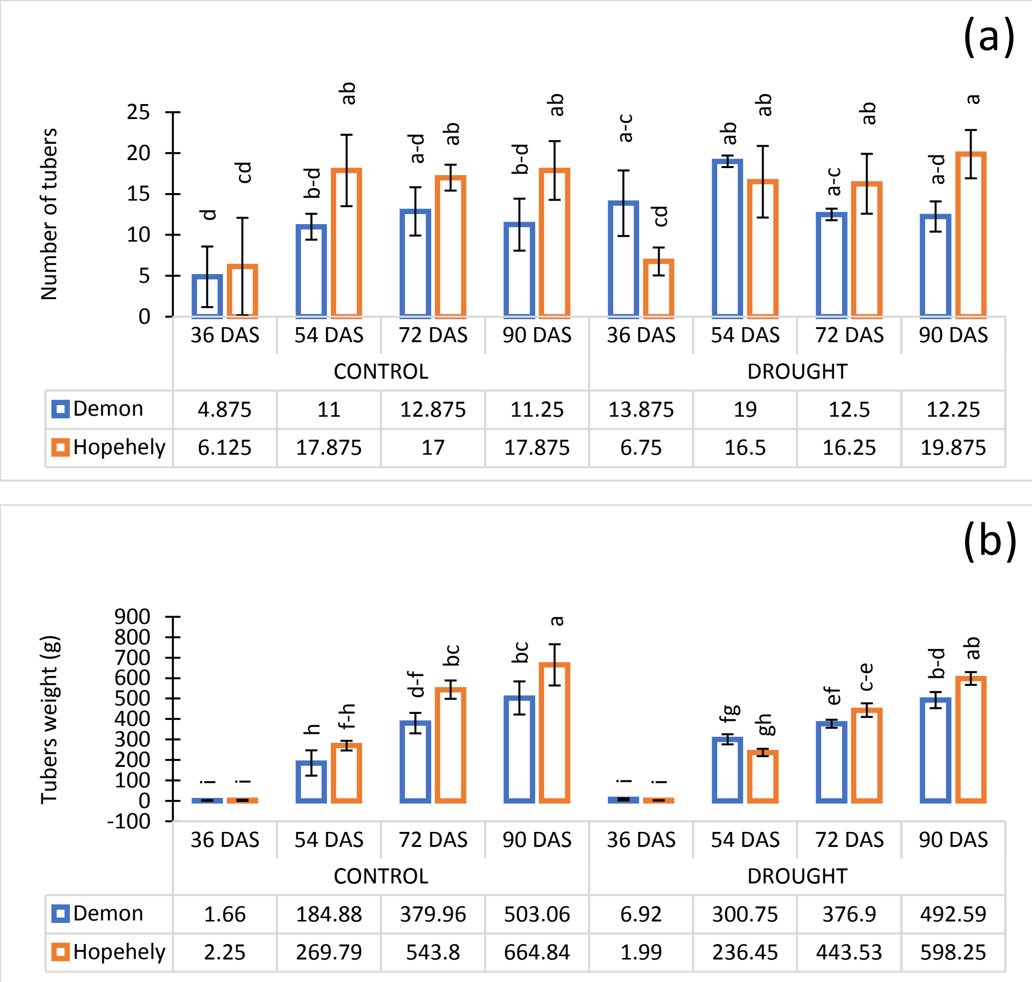


Figure 8 Effect of drought stress on: (a) number of tubers; (b) tuber fresh weight of potato genotypes. Each value is mean ± STDEV (standard deviation) of four replicates. Different letters indicate groups were significantly different ($p < 5\%$) according to Tukey's post-hoc test.

3.6 Correlation analysis

A Pearson correlation was computed to assess the linear relationship between variables. At early plant developmental stage (36 DAS) positive correlation was observed between foliage characteristics. Leaf area index positively correlated with leaf weight, shoot weight, number of leaflets, shoot length, leaf area ratio and total dry matter produced by plant. Correlation analysis at flowering stage (54 DAS) showed similar results. Foliage variables positively correlated with each other. There was a positive correlation between fresh plant weight and total dry matter, leaf weight, shoot weight, shoot length and leaf area index. At this stage, a negative correlation was observed between tuber yield and foliage characteristics (LAI, shoot length, specific leaf area, leaf weight and leaf area ratio). Tuber yield only corre (a) positively with number of tuber (b) this developmental stage.

At tuber bulking stage (72 DAS), positive correlation was observed between below ground parts. Tuber yield positively correlated with number of tubers, root fresh weight and dry weight, relative water content of leaves, leaf weight and nitrogen percentage in foliage. Foliage characteristics such as leaf weight also positively correlated with shoot weight, fresh plant weight., total dry matter, relative water content and nitrogen percentage in foliage. At senescence tuber yield positively correlated with (c) er of tubers and relative water co (d) of leaves. Root fresh weight also positively correlated with tuber yield and number of tubers (Fig. 9).

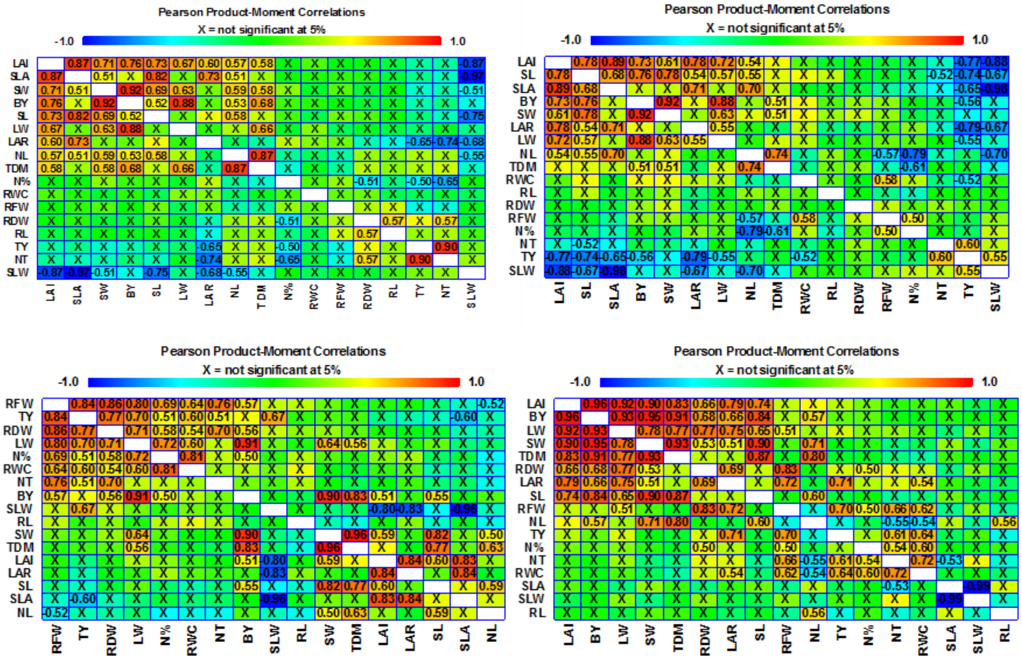


Figure 9 Pearson correlation among dependent variables at: (a) tuber initiation stage, (b) flowering stage, (c) tuber bulking stage, (d) Senescence. where, LAI: leaf area index; SLA: specific leaf area; SW: shoot weight; BY: fresh plant weight; SL: shoot length; LW: leaf weight; LAR: leaf area ratio; NL: number of leaflets per plant; TDM: total dry matter; N%: nitrogen percentage in foliage; RWC: relative water content; RFW: root fresh weight; RDW: root dry weight; RL: root length; TY: tuber yield; NT: number of tubers; SLW: specific leaf weight.

3.7 Discussion

Drought tolerance in plants is a complex mechanism based on several factors. However, several phenological parameters have been discussed in previous studies (Lahlou et al. 2003; Khan et al. 2015; Sprenger et al. 2016) to determine the drought tolerance ability of a plant. Potato genotypes vary in phenotypic response to drought stress that can help them in tolerating drought stress leading to a better-sustained yield. This study was conducted to describe which agro-physiological characters were the most related to yield and drought tolerance.

Reduction in plant height and leaf area is the first morphological symptom of drought stress in potatoes (Fleisher et al. 2008), followed by lesser canopy expansion and earlier senescence (Ahmadi et al. 2010; Obidiegwu et al. 2015; Oliveira et al. 2016). These results were confirmed in the present study where drought stress significantly reduced plant height, the number of leaflets per plant, and leaf area index. Early senescence under drought stress was also observed in 'Demon' that can be justified by the reduction in leaf area duration. Leaf size and retention account for LAI and LAD respectively, that directly affect tuber yield (Najm et al. 2010) and foliage dry matter (Chang et al. 2018) respectively. Moreover, LAI and LAD are more affected in later cultivars than earlier cultivars and have been reported as a major determinant of potato yield in previous studies (Gaur et al. 2017; Salavati et al. 2018; Pourasadollahi et al. 2019). Therefore, drought stress exhibited an inhibitory effect on the yield of potato genotypes by affecting growth and yield-related factors (Deblonde and Ledent 2001).

Between genotypes, 'Demon' and 'Hopehely' completely differed in plant establishment. Most of the above-ground characteristics i.e., plant height, number of leaves per plant, leaf rea duration were significantly better developed in 'Demon'. Taller plants, higher number of leaflets, and leaf area index directly contributed towards biomass production that in turn enhanced above-ground biomass of 'Demon', which on drying produced higher foliage dry matter (FDM) content. The increase in leaf number in 'Demon' was likely associated with the taller plant that provided with internodes elongation allowing more leaves from the apex of the plant to be exposed (Tadesse et al. 2001). Leaf size was another reason for the high leaf number in 'Demon' due to the size/number trade-off as smaller leaves are found on species that produce more of them. Westoby and Wright (2003) also reported a negative relationship between the number of leaves per shoot and individual leaf area. Relatively small leaves, because of higher heat exchange capacity are advantageous in hot, dry, high light, and low nutrient environments (Ackerly et al. 2002; Bragg and Westoby 2002; McDonald et al. 2003). It can be the reason for significantly higher leaf area duration in 'Demon' under control conditions. These results are in line with (Kebede et al. 2019) who showed genotypic differences in the ability to maintain leaf expansion with increasing soil moisture deficit.

Foliage dry matter being positively correlated with plant height and the number of leaves was also higher in ‘Demon’ (Masarirambi et al. 2012). The least tuber yield was observed for ‘Demon’ under drought stress conditions. It can be due to lower relative water content, nitrogen content and chlorophyll concentration that shows the inefficiency of ‘Demon’ plants to produce higher assimilates under drought stress. No correlation ($R^2 = 0.399$) was observed between above-ground parts and tuber yield that also justifies lower yield of ‘Demon’ despite significantly better vegetative growth. It showed that assimilates produced during vegetative growth were may be utilized in vegetative growth instead of tuber yield.

On the other hand, underground parts i.e., root fresh weight, number of tubers per plant, tubers weight per plant were significantly higher in ‘Hopehely’. ‘Hopehely’ developed a dense root system at the early stage of plant development that helped in better uptake and utilization of water and nutrients. Because of limited space available for root growth; no significant difference was observed for root length of the understudy genotypes but a significant difference was observed for root fresh weight that shows better uptake of water and nitrogen leading to better relative water content and nitrogen % in the foliage under both control and drought conditions. A greater and deeper root system is more drought-tolerant (Iwama 2008). Zarzyńska et al. (2017) reported that root density and LAI are determinant for yield. Besides a better and more developed root system; fewer and thicker leaves were also the reason for the high relative leaf water content in ‘Hopehely’ as the large leaf has fitness benefits derived from a greater boundary layer thickness for heat exchange, thus maximizing photosynthetic activity (Banik et al. 2016). A thick waxing layer on larger leaves reduces water losses thus maintaining higher leaf relative water content which is a drought tolerance characteristic (Vasquez-Robinet et al. 2008; Anithakumari et al. 2012). Higher chlorophyll concentration and nitrogen content in the foliage were also observed which shows a higher photosynthetic activity in ‘Hopehely’. Drought adaptation strategy in potatoes includes but is not limited to higher assimilate partitioning to tubers, producing larger tubers, or more tubers (Deblonde and Ledent 2001). ‘Hopehely’ produced heavier tubers indicating a high assimilate partitioning to tubers. However, under drought conditions, ‘Hopehely’ produced more tubers to ensure a better yield. Besides higher relative water content and higher root dry weight, an increase in antioxidant activity in ‘Hopehely’ also suggests a strong defence mechanism that can help in drought tolerance leading to higher yield (Mittler 2002; Yasmeen et al. 2013). Yang and Poovaiah (2000) also reported that SOD and CAT scavenge reactive oxygen species produced under stress conditions that help the plant to tolerate stress.

4 NEW SCIENTIFIC RESULTS

1. We observed that two Hungarian potato genotypes, namely 'Demon' and 'Hopehely' varied significantly in their plant development and their response to drought stress. 'Demon' showed a decrease in vegetative growth under drought stress compared to control conditions while 'Hopehely' maintained its vegetative growth.
2. It was also established that leaf area duration is more closely related to potato yield than leaf area index in the case of these two genotypes studied.
3. Relative water content (RWC) confirmed as an indicator of drought tolerance in the studied potato cultivars.
4. Both genotypes produced higher number of tubers to maintain yield under drought stress.
5. Based on the results of experiment, 'Hopehely' is recommended to be used under water limited conditions.

5 LIST OF PUBLICATIONS

1. Nasir, M.W.; Toth, Z. 2022. Effect of Drought Stress on Potato Production: A Review. *Agronomy*, *12*, 635. https://doi.org/10.3390/agronomy12030635
2. Nasir, M.W., and Toth, Z. 2021. Effect of drought stress on morphology, yield, and chlorophyll concentration of Hungarian potato genotypes. Journal of Environmental & Agricultural Sciences. 23(3&4): 8-16.
3. Nasir, M.W.; Toth, Z. 2021. Response of Different Potato Genotypes to Drought Stress. Agriculture, 11, 763. https://doi.org/10.3390/agriculture11080763
4. Nasir, M.W., M.R. Ali, M. Imran, M. Avezbayev, and M. Ebubekir. 2020. Response of *Triticum aestivum* L. to exogenous application of plant growth regulators. Georgikon for Agriculture, Vol. 24, No. 4, 117-132.
5. Nasir, M.W., A. Yasmeen, M. Imran and T. Zoltan. 2019. Seed priming to alleviate drought stress in cotton. Journal of Environmental & Agricultural Sciences 21:14-22

Abstracts published in international conferences

1. **Muhammad Waqar Nasir**, Azra Yasmeen, Muhammad Imran, Hassan Nawaz and Zoltán Tóth; Effect of Heat Stress and Its Mitigation Strategies by Seed Treatment and Foliar Application in Wheat (*Triticum Aestivum L.*). In: 1st Aus-Pak International conference on wheat for food security; 2019, March 24-25; MNS University of Agriculture, Multan, Pakistan. pp. 48
2. Muhammad Imran, Azra Yasmeen, **Muhammad Waqar Nasir** and Hassan Nawaz; Sewage water: promoter or inhibitor for growth and yield in maize (*Zea mays* L.). In: M.I.A Rehmani, J. Iqbal and S. Bashir (Ed). 1st International conference on Sustainable Agriculture: Food security under changing climate Scenarios; 2019, April 03-05; Ghazi University, DG Khan, Pakistan. pp. 177
3. **Muhammad Waqar Nasir**, Azra Yasmeen, Muhammad Imran, and Zoltán Tóth; Use of seed priming to mitigate drought stress in cotton. In: G. Pintér. S. Csányi and H. Zsiborács (Ed.) 61st Georgikon conference on Innovation challenges in the 21st century; 2019, October 03-04; Georgikon Faculty, University of Pannonia, Keszthely, Hungary. (https://napok.georgikon.hu/hu/)

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