Impact of water deficit on growth, productivity, and water use efficiency in potatoes (*Solanum tuberosum* L.)

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# Abstract

Drought stress, a serious constraint affecting yields of almost all major crops, is expected to get worse with water deficits stemming from global climate change and human population expansion. Modern cultivars of potato (*Solanum tuberosum* L), the fourth most important food crop worldwide, are highly sensitive to drought stress. We evaluated a range of agro-physiological traits of fifteen potato genotypes under well-watered (WW) and water deficit (WD) conditions to understand the impact of drought stress on potato productivity and to identify traits for selection of drought tolerant potato genotypes. Our results showed that the drought stress tolerant genotypes, CIP392797.22 (UNICA), CIP397077.16, CIP398190.89, CIP398208.219 and CIP398208.620, were able to preferentially put limited water toward tuber production rather than biomass. These genotypes have high tuber production under WW conditions and increased photosynthetic activity and water use efficiency under WD. Variables such as harvest index, root dry weight, relative chlorophyll content and chlorophyll concentration can be used to select drought tolerant potato genotypes in breeding programs.

**Key words:** abiotic stress, harvest index, physiological traits, drought tolerance, SPAD

# Introduction

Water has become a scarce and precious resource and its efficient utilization in food production is a key challenge worldwide. Specifically, yield losses due to drought are a global problem limiting agricultural production (Obidiegwu et al., [2015](#ref-obidiegwu2015Coping)). Tracking the consequences of drought stress in plants is a difficult task, as it depends on the timing, intensity, type of crop, and duration of stress (Blum, [2011](#ref-blum2011Drought)). Potato (*Solanum tuberosum* L.), the fourth most important food crop worldwide with an annual production of around 380 million tons, suffers drastic losses in tuber yield and/or quality due to drought stress (Stark et al., [2013](#ref-stark2013Potato); Yang et al., [2016](#ref-yang2016Identification)). In general, potato has a high harvest index (HI) in comparison with cereals and relatively low demand for water *i.e.*, 400 to 600L for 1kg of tuber dry matter (Monneveux et al., [2013](#ref-monneveux2013Drought); Sprenger et al., [2016](#ref-sprenger2016drought); Stark et al., [2013](#ref-stark2013Potato)). However, potatoes are sensitive to drought stress because of their shallow root system, and fast closure of stomata, which reduces transpiration and photosynthesis, considerably reducing tuber yields (Deblonde & Ledent, [2001](#ref-deblonde2001Effects); Joshi et al., [2016](#ref-joshi2016Potato)). Periodic water shortages are critical, particularly at the tuber initiation stage. Therefore, potato cultivars with effective water-saving mechanisms leading to higher tolerance are highly desirable for semi-arid areas (Monneveux et al., [2014](#ref-monneveux2014Drought)). The majority of modern potato cultivars are very sensitive but with variable responses to drought stress (Monneveux et al., [2013](#ref-monneveux2013Drought); Soltys-Kalina et al., [2016](#ref-soltys-kalina2016effect); Sprenger et al., [2016](#ref-sprenger2016drought)). Drought stress tolerance in potatoes is a complex trait controlled by a large number of minor effect QTLs (quantitative trait loci). Significant QTLs and differentially expressed genes under drought stress have been identified in potato (Khan et al., [2015](#ref-khan2015Multiple); Anithakumari et al., [2012](#ref-anithakumari2012Genetic); Watkinson et al., [2006](#ref-watkinson2006Accessions); Chen et al., [2020](#ref-chen2020Transcriptome)). Also, wild species and potato cultivars have been shown to vary in morphological and physiological traits as well as biochemical and molecular pathways under drought stress (Liu et al., [2005](#ref-liu2005ABA); [2006](#ref-liu2006Effects)).

In Peru, the center of origin of potatoes, landraces and wild potato relatives have great diversity for physiological traits desirable for breeding potatoes with enhanced drought tolerance. Water use efficiency (WUE), the efficiency of the amount of water applied and used for transpiration that goes toward dry matter production, is an important trait for adaptation to drought stress. Enhanced WUE can reduce crop water requirements and significantly increase crop yield (Tolk and Howell 2009). When a higher WUE under drought stress is maintained, the effects of water deficiciency are reduced and the competitiveness for water under drought conditions is enhanced (Ogaya and Peñuelas 2003). WUE was found to be greater in the summer due to the greater HI and more-efficient interception of solar radiation per unit of applied water by drought-exposed than by well-watered plots (Trebejo and Midmore 1990). Cultivars can be bred for WUE, increasing yield per unit of water, by capturing traits that help the plants develop faster, flower earlier, have a lower leaf area index, and be more efficient in capturing radiation (Blum 2005; Hochman et al. 2009). Cultivars with low stomatal conductance (gs) during vegetative stages, higher transpiration efficiency (TE), and an improved relationship between dry matter produced and the quantity of soil water utilized, can ensure good tuber yield and quality under drought stress (Condon et al. 2004; Carli et al. 2014). In normal conditions when irrigation is sufficient to meet the transpiration needs of the crop, genotypes with higher stomatal conductance and low WUE and thus able to extract more water from the soil will have higher yields. In contrast, when water is not sufficient, the low WUE arising from a high rate of transpiration leads to low yields in favor of dry matter partitioning to reproductive organs (Tuberosa 2012).

The aim of the present study is to understand the mechanisms for drought tolerance and growth, physiological, and yield responses in fifteen potato genotypes under water deficit conditons. In addition, the relationships between different agro-physiological, root traits, and yield under water-limited conditions were explored and traits to select drought tolerant potato genotypes were identified.

# Materials and Methods

***Plant material and experimental design***

Two commercial varieties and thirteen potato genotypes from the advanced breeding population at the International Potato Center (CIP) were used in this study (Table 1). The commercial varieties were: UNICA (CIP392797.22) with a good yield in warm and dry environments (Demirel et al., 2020; Gutiérrez-Rosales et al., 2007; Rolando et al., 2015); and Achirana INTA (CIP720088) known for its earliness and drought tolerance (Schafleitner et al., 2007). The plants were grown in a controlled greenhouse at 28/15°C day/night with 70±5% average relative humidity and had a weather station ‘HOBO U12 Outdoor/Industrial model’ (Onset Computer Corporation, Bourne, MA, USA).

The potato tubers were pre-sprouted for 2 weeks in dark chamber before planting. Afterwards, one tuber/genotype was sown at at 5–7cm depth in a 5L plastic pot containing 5kg of dry commercial Sogemix SM2 (75% Peat Moss, perlite, vermiculite, and limestone). Fertilization was done twice during the experiment with ammonium nitrate; triple superphosphate and potassium sulphate, one before planting (mix with the substrate) and the other applied at the surface at 40 DAP (Days after planting).

## Irrigation treatments

The experiment was carried out in a complete randomized block design with two irrigation treatments and had 5 replications of each genotype per treatment. In well-watered (WW) treatment, plants were irrigated according to their transpiration demand (Figure 1B) and in water deficit (WD) treatment, water supply was gradually reduced until wilting. At 35 DAP, prior to the stress initiation, the pots from both WW and WD treatments were watered to soaking and then allowed to drain overnight. Next morning, the pots were sealed in a plastic bag secured with a twist tie to prevent water loss except by transpiration. Thereafter, all the pots were weighed and this weight was defined as the initial pot weight. The inter-daily weight of the pots was measured for 10 days to calculate the initial dry down parameters for treatment application (Figure 1A). WD treatment was imposed at 45 DAP to coincide with the beginning of tuber initiation and water was reduced by 150 mL in each irrigation.

## Transpiration rate

The transpiration rate of each plant was calculated by the procedure previously described by Bhatnagar-Mathur et al. ([2007](#ref-bhatnagar-mathur2007Stressinducible)) and Ray & Sinclair ([1998](#ref-ray1998effect)). Transpiration was calculated by weighing the pots every two days in the afternoon between 13:00 and 15:00 hours (GMT -05:00), subtracting the amount of water added, and calculating the difference in weight between two days. On average, a total of 275.69 ml and 72.51 ml of water were added to each pot in WW and WD treatment (Figure 1B). The inter-daily transpiration rates of WD plants were normalized against WW plant rates to reduce the influence of day-to-day variation, as follow, . The normalization was achieved by dividing transpiration of each individual plant in the WD regime by the mean transpiration of the WW plants. For comparing the transpiration between plants, a second normalization was done so that the normalized transpiration rate (NTR) of each plant was defined as 1.0 when the soil water content in each pot was at field capacity (Sinclair & Ludlow, [1986](#ref-sinclair1986Influence)). The available soil water or the fraction of transpirable soil water (FTSW), for each pot was calculated by dividing the pot weight minus the final pot weight by the transpirable soil water of that pot, using the following formula, .

## Trait evaluation

**Water Use Efficiency (WUE)**

We have calculated the biomass water use efficiency (wueb) and tuber water use efficiency (wuet). The wueb was calculated as the total biomass in dry weight (unit?) produced divided by the cumulative water transpired (Costa et al., [1997](#ref-dallacosta1997Yield)); for wuet we used the dry weight (unit?) from tuber production divided by the total water transpired (mL) during the treatment.

**Relative Water content (RWC)**

Relative water content (RWC) was determined by weighing the 3rd leaflet from the youngest fully expanded leaf (third leaf from the apical part) of each plant (FW), and then placing it in a 4x3 inch Ziploc bag containing distilled water for 24 hours. Excess water was removed by blotting each leaf in a paper towel prior to taking turgid weight (TW) and afterwards dried in an oven overnight at 90 ºC. After drying, leaves were reweighed (dry weight, DW). RWC was calculated following the formula described by Vasquez-Robinet et al. (2008).

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**Leaf Osmotic Potential (LOP)**

Leaf Osmotic Potential (LOP) was determined in leaf discs of 5mm diameter taken from the third fully extended leaf. Leaf discs were put in 1 ml cryogenic tubes and frozen in liquid nitrogen for further analysis. Before taking readings, the frozen leaves were incubated at 22°C for 30 min in a sealed C-52 chamber (Wescor Inc., Logan, UT, USA). Osmotic potential was determined using a dew point microvoltmeter (HR-33T from the same company). The degree of total osmotic adjustment (OA) was defined as the difference in OP between the WW and the WD plants (Hessini et al., 2009).

**Chlorophyll content (SPAD)**

Chlorophyll content of leaves was evaluated by taking SPAD measurements using a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) from the youngest fully expanded leaf (third leaf from the apical part), from three points (upper, middle and lower leaflet of a leaf). Individual readings of leaflets were averaged to represent individual measurement of a leaf. Measurements were done on light adapted leaves at 29, 59, 76, and 83 DAP. SPAD measurements of the leaf were used as an indicator of nitrogen status and leaf senescence.

**Post-harvest traits**

At harvest (90 DAP), four components were separated out: leaves, stems, roots and tubers and used to determine total leaf area (cm2), fresh weight (FW), and dry weight (DW) of leaves, stems, roots, and tubers. Dry weight was determined by drying all the components at 80 °C for 3 days in a forced air oven. The leaf area (cm2) of the plants was measured by taking photographs of all the leaves arranged on a wooden board and analyzing the images using using ImageJ software (Rueden et al., 2017; Zárate-Salazar et al., 2018).

**Indices**

The harvest index (HI) was calculated as the ratio of TDW related to the total dry biomass (TDB; g) and specific leaf area (SLA) was calculated by dividing the leaf area (LFA) with lead dry weight (LDW). Relative chlorophyll content (rcc) was calculated with the relation between SPAD at 83 dap (SPAD\_83) and leaf area (LFA) multiplied by 100 for better scale interpretation.

## Statistical analysis

Statistical analysis was performed with the statistical software R (R Core Team, [2019](#ref-R-base)). The analysis of variance (ANOVA) was performed to evaluate the differences between the factors and the comparison of the means with the Student-Newman-Keuls test (*p*<0.05) with agricolae and GerminaR package (de Mendiburu, [2020](#ref-R-agricolae); Lozano-Isla et al., [2019](#ref-lozano-isla2019GerminaR)). A Student’s t-test was performed between WW and WD treatment (*p*<0.05). Multivariate analysis, correlation and principal components analysis (PCA) were performed with FactoMineR and heatmaply packages (Galili et al., [2018](#ref-galili2018heatmaply); Husson et al., [2020](#ref-R-FactoMineR)). Euclidean distance was used for computing hierarchical clustering between treatments and genotypes (Lê et al., [2008](#ref-le2008FactoMineR)). For reproducible analysis, the code and statistical analysis used in this manuscript are available in the following GitHub repository: <https://github.com/flavjack/20130515LM>.

# ResultS

## Impact of treatments on soil water supply and transpiration

The fraction of transpirable soil water (FTSW) differences among treatments every 4 days and inter-daily transpiration rates (TRS) in potato genotypes were recorded. The FTSW in WW plants was maintained above 70%, while for the WD treatment the gradual restriction in water supply decreased the water availability. By the end of the experiment the plants in WD had less than 10% of FTSW (Figure 1A). In the case of the transpiration rate, the reduction in the plants in WD was visible after 8 days of water restriction (Figure 1B).

## Agro-physilogical traits

The chlorophyll concentration (SPAD units) taken over the course of plant development (29, 59, 76, and 83 DAP) showed that at 29 DAP all plants were at the same stress level (Figure 2E). By the end of the experiment, difference between treatment (T), genotypes (G), and G\*T were found and the values were lower than at 29 DAP (Table S1). SPAD values in WD treatment for all the genotypes were higher than the ones at WW conditions (Table 2). The genotypes CIP398190.89 and CIP720088 had the lowest differences for SPAD at 83 DAP among treatments (2.06 and 0.30%, respectively), while CIP398203.244 and CIP398208.33 had the largest (14.48 and 17.54%, respectively) (Figure 2F).

Leaf relative water content (RWC) and leaf osmotic potential (LOP) significantly (*p*<0.01) decreased in response to WD in all the genotypes (Table 2). The values ranged between 64.96% and 50.09% for CIP720088 and CIP398201.510, respectively. The specific leaf area (SLA) reduction was 48% under WD treatment compared to WW (Table 2). CIP398190.89 together with CIP398203.5 were among the clones with lowest reduction (24 and 21% respectively), while CIP398208.219, CIP398098.119, and CIP398208.704 were among the clones with highest SLA reduction (53, 65, and 64% respectively).

Plant hight (HGT), leaf dry weight (LDW), stem dry weight (STD), and leaf area (LFA) decreased significantly (*p*<0.01) under drought treatment (Table 2). In the case of LFA, there was a drastic reduction of 65% in plants under WD treatment compared to WW plants (Table 2 and Figure 2B). While the components such as number of tuber (NTUB), root dry weight (RDW) and root length (RDL) did not shown significant differences between the treatments.

The relative chlorophyll content (RCC), the relation between the chlorophyll concentration in the leaves (SPAD) in relation with the leaf area, has been shown significant (*p*<0.001) difference between treatments. RCC was able to discriminate genotypes under WW and WD treatments (Figure 2B). The genotypes with best performance for RCC were CIP720088 (Achirana-INTA), CIP398208.620, CIP398208.704, CIP398201.510, CIP392797.22 (UNICA) and CIP397077.16 (Figure 3B).

## Yield components

Large differences existed among genotypes in total dry biomass (TDB) of genotypes under WW treatment at the end of the experiment (Table 2, Table S1). WD treatment had a significant effect (*p*<0.001) with an average reduction of around 32% in comparison with the WW treatment (Table 2). In terms of productivity, WD treatment decreased tuber yield (TDW) across genotypes by an average of 40% (*p*<0.001). CIP398190.89 had greater tuber dry weight (g) in WD treatment compared to its yield in WW treatment with a 5% increase in biomass, while others genotypes like CIP398203.5 and CIP398203.244 presented up to 56% and 48% reduced tuber production (Figure 2A). The genotypes CIP398203.244, CIP398180.612, and CIP398201.510 were among the most sensitive genotypes at 31.56, 46.75 and 48.88% respectively under WD. Significant differences were also found among genotypes (*p*<0.001) and treatments (*p*<0.02) for harvest index (Table 2).

Biomass water use efficiency (WUEb) was generally higher for genotypes under WD treatment than WW treatment (*p*<0.001) (Table 2). Under WD treatment, CIP397077.16, CIP398208.620, CIP392797.22 and CIP398190.89 showed the highest WUEb with 13.06, 12.03, and 11.59 gL-1, respectively (Figure 3A). The lowest WUEb with 8.50 and 9.24 gL-1 were presented by CIP398180.612 and CIP398203.5, respectively. For tuber water use efficiency (WUEt) there is no significant difference between treatments (Table 2). The genotypes with better WUEt under WD treatment were CIP397077.16, CIP392797.22, CIP720088 and CIP398208.620 (Figure 1 and 2D).

## Multivariate analysis

The Principal Component Analysis (PCA) identified the first two dimensions explaining 64.9% of the variance in the experiment (Figure S2A). In the first dimension the variables with highest contribution are TRS (r=0.94), LFA (r=0.93), TBD (r=0.87), SLA (*r*=0.81), TDW (r=0.74) and LDW (r=0.73) (Figure S2B). These variables showed a high correlation among each other under WW treatment. On the other side, RCC (*r*=-0.73), and SPAD (r~-0,63) showed negative correlations and are associated to WD treatment (Figure S2D). In the second dimension the variables with major contribution are RDL (*r*=0.88) and RDW (*r*=0.87), with a high correlation under WW treatment and negative correlation with HI (*r*=-0.92) and WUEt (*r*=-0.90) under WD treatment (Figure S2C-D).

PCA showed five clusters of genotypes (Figure 3B). These groups were separated by the treatment applied. Genotpes in the cluster 4 and 5 are under WW treatment, and the cluster 2 and 3 had genotypes under WD treatment. The genotypes in the cluster 1, 3 and 5 can tolerate water stress and have shown a significant correlation with WUEt, HI and TDW, traits that are important in the yield component (Figure 3B). The relationship between the clusters and variables shown that the genotypes in the cluster 2 are positively correlated with the SPAD, RDL and RDW and negative correlated to TDW, HI and WUEt. The genotypes in the cluster 3 and 1 are positively correlated with the RCC and WUEb. In the cluster 4, the genotypes are positively correlated with LDW, TDB, LFA and TRS. The genotypes in cluster 5 are correlated with SLA, TDW and RWC and negative correlated withSPAD and RDW (Figure 3).

According the Pearson correlation analysis (Figure S4) values of TDB and TRS were strongly and positively correlated with 0.93 (*p*<0.05) with Euclidean distance of 0.91 (Figure S4). The SPAD measurements shown strong correlation between them (r~0.80) and at negative correlation with HI (r=-0.07). A negative correlation (r= -0.73) among RDW and HI was found with a Euclidean distance of 4.96. A strong correlation between HI and WUEt was found (r=0.92) with an Euclidean distance of 0.68 (Figure S4). Tuber dry weight (TDW) showed correlation with HI (r=0.61) and WUEt (r=0.55) and WUEb (r=0.05) with a Euclidean distance of the traits 4.96 (Figure 3 and S4). We also found correlation between HI and WUEt was 0.92 with a Euclidean distance 0.91 and a negative correlation with spad (-0.7) at 38 DAP.

The negative (r~0.62.7) correlation (Table 2) and Euclidean distance of 4.21 between SPAD and TDW since the application of the drought treatment indicates no association between them. Apparently SPAD measure is sensitive to detect the drought stress and it is related with the tuber production (Figure 3 and S4). The inclusion in the analysis of the relative chlorophyll content (RCC) was able to differentiate the genotypes by their photosynthetic and water use efficiency (Figure 3 and 2B).

# Discussion

Water deficit (WD) triggered a range of morphological and physiological mechanisms in the potato genotypes tested, leading to different yield penalties and indicating a variety of survival strategies by potato. We found root length, SPAD, and SLA to be good indicators for water use efficient (WUE) plants useful for drought tolerance breeding. Most characteristics measure showed differences between treatments, pointing to their value in evaluating the impact of drought. According to (Songsri et al. 2009), enhanced extraction of water and nutrients from the soil due to large root system and long roots is a drought resistance mechanism in potato. Our study showed that the minimal yield losses in some genotypes like CIP398190.89 under WD could be related to its ability to increase its WUE, by absorbing the limited water and nutrients available in the soil without changing its morphology. However, in our study, the genotype with the longest roots (CIP398201.510) showed the lowest yield and HI under both stressed and non-stressed conditions. In this genotype, the long roots seem to contribute to vegetative growth rather than harvestable yield.

Another drought resistance mechanism can be the reduction of transpiration achieved by the reduction of leaf area, for example, thick leaves often have greater photosynthetic capacity than thin leaves, due to their higher chlorophyll per leaf area counts (Songsri et al. 2009). SPAD is an indicator of the photosynthetically active light transmittance characteristics of the leaf, which is dependent on the unit amount of chlorophyll per unit leaf area. SPAD units have been correlated with chlorophyll and carotenoid content in potato and other crops (Ramírez et al. 2014: Marenco et al. 2009) and are used in selecting genotypes tolerant to drought in breeding programs. In this study, some genotypes reduced specific leaf area (SLA), and changed the ratio of root to shoot, while others, like CIP3977077.16 and CIP398190.89 maintained SLA, as well as harvest index (HI), root length and stolon mass under WD. These genotypes may have lower sink competition and allocated a proportionally higher fraction of assimilates to tubers by reducing the evaporative surface area above-ground and efficiently employing the resources (water saved and N) located in the soil. Conversely, sensitive genotypes such as CIP398203.244 preferred to produce leaves and stems in preference to tubers. Our study also found a lower specific leaf area (SLA) under WD, principally explained by decreases in new leaf production, number of leaves, and leaf size. This decrease might have had allowed a reduction in leaf transpiration, saving water for tuber bulking as suggested by Lahlou, et al. (2003). Hence, potato genotypes with the ability to maintain higher SPAD and lower SLA under WD conditions, can maintain higher WUE under WD conditions.

It is important to remember that under WW conditions, soil can supply water at a steady rate to meet the transpiration demand. However, as the soil becomes dry, water flux from soil to root surface decreases and cannot satisfy the demand of transpiration. Additionally, in WD conditions, mineralization and supply of nutrients, especially N, is reduced, forcing the plant to use their resources efficiently as the only solution to not compromise yield (Motalebifard et al. 2013). Payne et al. (1995) found that N uptake was linearly related to plant transpiration, and that about twice as much N is taken up per kg of transpiration in water-stressed plants than in non-water-stressed plants. In our study, we found that CIP397077.16 and CIP398208.620 could maintain turgor, which, combined with the reduction of SLA, helped them to conserve water. Additionally, increased amounts of RWC under WD treatment in CIP720088 may have been the result of higher stomata conductivity, since the root length of CIP720088 in WW and WD treated plants were comparable.

The strong correlation between WUE in this experiment and tuber yield (*r = 0* .78, *p <* .001) indicates direct association between them, and at the same time, reflected the conservative relationship between biomass production and WUE through a range of limited soil water availability. Reduced stomatal conductance can constrain the diffusion of CO2 for photosynthesis, and accordingly the average total biomass in the WD treatment was significantly lower than that of the corresponding genotypes in WW treatment. Different studies have measured WUE at the leaf, whole plant, and crop levels (Guoju et al. 2013; Hochman et al. 2009) and have found no significant differences at plant or crop levels (Deblonde et al. 1999). In this study, WUE is considered a critical trait that can affect yield and its increase can lead a plant to tolerate or avoid water deficit conditions.

Based on our results, the genotypes with reduced SLA, longest root length and that maintain relatively high WUE under WD conditions, like CIP398201.510, are drought avoidant. Additionally, we found that tolerant genotypes like CIP397077.16, CIP398190.89, and UNICA preferentially use available water for tuber production rather than above ground biomass. Our study demonstrated that root length, SPAD and SLA are important traits related to WUE and could be useful as selection criteria of new genotypes. However, there are additional strategies of the below-ground plant parts that need to be explored for their role in yield stability under water stress conditions.

~~In field conditions the abiotic stress is a combination of several factors and not only drought, for this reason, study the physiological mechanisms of tolerance to water stress in potato under control conditions is necessary (Kaminski et al.,~~ [~~2015~~](#ref-kaminski2015Contrasting)~~; Zegada-Lizarazu & Monti,~~ [~~2013~~](#ref-zegada-lizarazu2013Photosynthetic)~~). We applied a water deficit experiment with gradually reduction of the water supply and evaluate the process in fifteen genotypes (Figure 1). Most characteristics measure showed differences between treatments, pointing to their value in evaluating the impact of drought (Table 2). As potato is a tuber crop, the interaction of the variables underground are important because exist a differentiation of the architecture of root system under field condition (Zarzyńska et al.,~~ [~~2017~~](#ref-zarzynska2017Differences)~~). According to Songsri et al. (2009), enhanced extraction of water and nutrients from the soil due to large root system and long roots is a drought resistance mechanism in potato. In our work we did not find difference in root length and root dry weight (Table 2) maybe because root development in all cultivars was limited by the volume of the pots, in which plants were grown (Soltys-Kalina et al.,~~ [~~2016~~](#ref-soltys-kalina2016effect)~~).~~

~~While plant biomass accumulation and yield was shown to be inextricably linked to transpiration (Sinclair et al.,~~ [~~1984~~](#ref-sinclair1984WaterUse)~~) (Figure S4). Genotypes shown harvest index around 53% under WW condition with a reduaction at 11% in WD (Table 2), similar results were found for Schafleitner et al. (~~[~~2007~~](#ref-schafleitner2007Field)~~) with 14% under terminal drought. In comparison with other tuber crops with reductions until 57% under water stress (Ruttanaprasert et al.,~~ [~~2016~~](#ref-ruttanaprasert2016Effects)~~), potato shown moderate reduction in this trait and is considered to use water more efficiently than cereals (Shahnazari et al.,~~ [~~2007~~](#ref-shahnazari2007Effects)~~). Under WD the genoypes presented strong reduction in the leaf area (Table 2) that is consider one of mechanism to drought resistance to reduce the transpiration with thick leaves which often have greater photosynthetic capacity than thin leaves (Figure 2B), due to their higher chlorophyll per leaf area (Rolando et al.,~~ [~~2015~~](#ref-rolando2015Leaf)~~; Songsri et al.,~~ [~~2009~~](#ref-songsri2009Association)~~). SPAD units have been correlated with chlorophyll, carotenoid, nitrogenous status and crop senescence in potato and used to select tolerant genotypes under drought condition (Ramírez et al.,~~ [~~2014~~](#ref-ramirez2014Chlorophyll)~~; Rolando et al.,~~ [~~2015~~](#ref-rolando2015Leaf)~~; Saravia et al.,~~ [~~2016~~](#ref-saravia2016Yield)~~; Silva-Pérez et al.,~~ [~~2020~~](#ref-silva-perez2020Genetic)~~). Our results shown that SPAD measurement are sensitive to detect water stress in potato. Before the treatment application the genotype did not presented difference in the SPAD (Figure 2E), but since the plants had a water shortage the spad content show differences between WW and WD plants (Table Table 2, Figure S4 and 2F). The SPAD content also present a negative correlation with the TDW (Figure S4). Genotypes under drought stress try to increase their SPAD content (Figure 3) (Rodríguez-Pérez et al.,~~ [~~2017~~](#ref-rodriguez-perez2017Drought)~~) for offset the reduction in the leaf area because the impose of drought decrease severely the leaf area and loss of water turgor (Table 2) (Ramírez et al.,~~ [~~2014~~](#ref-ramirez2014Chlorophyll)~~; Rolando et al.,~~ [~~2015~~](#ref-rolando2015Leaf)~~). This phenomenon causes that the plants try to be more efficient with less leaf area and increase the photosynthetic activity (Figure 2F). For compare this interaction, we introduced an index denominated relative chlorophyll content (rcc) as the quantity of chlorophyll content related to their leaf area. Genotypes with the ability to maintain high relative chlorophyll content under WD conditions, can maintain higher wue~~~~t~~ ~~and hi, reducing the losses in the tuber production are good candidates under drought condition (Figure 3).~~

~~It is important to remember that under WW conditions, soil can supply water at a steady rate to meet the transpiration demand. However, as the soil becomes dry, water flux from soil to root surface decreases and cannot satisfy the demand of transpiration. Additionally, in WD conditions, mineralization and supply of nutrients, especially N, is reduced, forcing the plant to use their resources efficiently as the only solution to not compromise yield (Motalebifard et al. 2013). In our study, we found that CIP392797.22 (UNICA) and CIP397077.16 were clustered between the good performed genotypes with high wue~~~~t~~~~, hi and tuber production with no difference under WW and WD condition (Figure 3 and 2). The same genotypes under field condition were reported tolerance to drought (Saravia et al.,~~ [~~2016~~](#ref-saravia2016Yield)~~). We found strong relation between hi and wue~~~~t~~ ~~(Figure 3A and S1). Since hi has been found to be stable for a particular cultivars over wide range of conditions (Donald & Hamblin,~~ [~~1976~~](#ref-donald1976Biological)~~; Khan et al.,~~ [~~2015~~](#ref-khan2015Multiple)~~). For this reason obtaining high harvest indexes under water-limited conditions is especially important to obtain high water-use efficiency genotypes (Passioura,~~ [~~1977~~](#ref-passioura1977Grain)~~), even if in many crops it appears unlikely that substantial improvements in harvest index could be achieved (McVetty & Evans,~~ [~~1980~~](#ref-mcvetty1980Breeding)~~). Minimal yield losses in some genotypes like CIP398190.89 (Figure 3B) under WD could be related that it is the unique genotype with early maturity and its ability to increase their rcc under a reduced leaf area resulting in a better performance in wue~~~~t~~ ~~using the limited water and nutrients available in the soil without a drastically reduction in the final yield (Figure 3, 2A, S3). For this reason, genotypes that shown increase in their relative chlorophyll content (rcc) under drought stress were associated with better water use efficiency as mechanisms to mitigate the yield reduction (Figure 3A-B). Genotypes under WW and WD condition such as CIP392797.22 (UNICA), CIP397077.16, CIP398190.89, CIP398208.219, CIP398208.620 shown good response in tuber production under WW condition as well as they have better water use efficiency and harvest index for tuber production under WD (Figure 3). In previous works, Saravia et al. (~~[~~2016~~](#ref-saravia2016Yield)~~) presented that two of these five genotypes, CIP392797.22 (UNICA) and CIP397077.16 were tolerant to drought in terms of yield maintenance and used the nitrogen present in the soil more efficiently. Sensitive genotypes such as CIP398203.244 and CIP398201.510 preferred to produce leaves and more roots instead of tubers (Figure S6). In these genotypes more roots seem to contribute to vegetative growth rather than yield components in contradiction to Songsri et al. (~~[~~2009~~](#ref-songsri2009Association)~~) who mention that enhanced extraction of water and nutrients from the soil due to large root system and long roots is a drought resistance mechanism in peanut. However, a deeper and more extensive rooting system may have drawbacks, because a greater root biomass would almost surely result in a low harvest index in potato (Figure 3A).~~

~~The present work gives an overview of the behavior of different potato genotypes under water deficit condition and the penalty for the yield causes by the water shortage. Most measured characteristics shown differences between treatments, pointing to their value in evaluating the impact of drought. Uni-variate analysis shows that it is limited to understand the response of the potato under drought stress. Tolerant genotypes like CIP392797.22 (UNICA), CIP397077.16, CIP398190.89, CIP398208.219, CIP398208.620 preferentially use available water for tuber production rather than biomass production represented by their high wue~~~~t~~~~. The genotypes with high harvest index and relative chlorophyll content present mechanisms for drought avoiding as they present good performance in tuber water use efficient. A fast screening traits would be helpful in selecting valuable genotypes with defined growth strategies that translate to drought tolerance and are suitable for experiments and/or breeding (Soltys-Kalina et al.,~~ [~~2016~~](#ref-soltys-kalina2016effect)~~). Variables such as harvest index and SPAD are outlined to be a good indicators for tuber water use efficient and useful traits for direct and indirect selection as selection criteria in drought stress experiments, using fast, easy and inexpensive evaluations for first stage of breeding programs in potato where is required to evaluate large populations.~~

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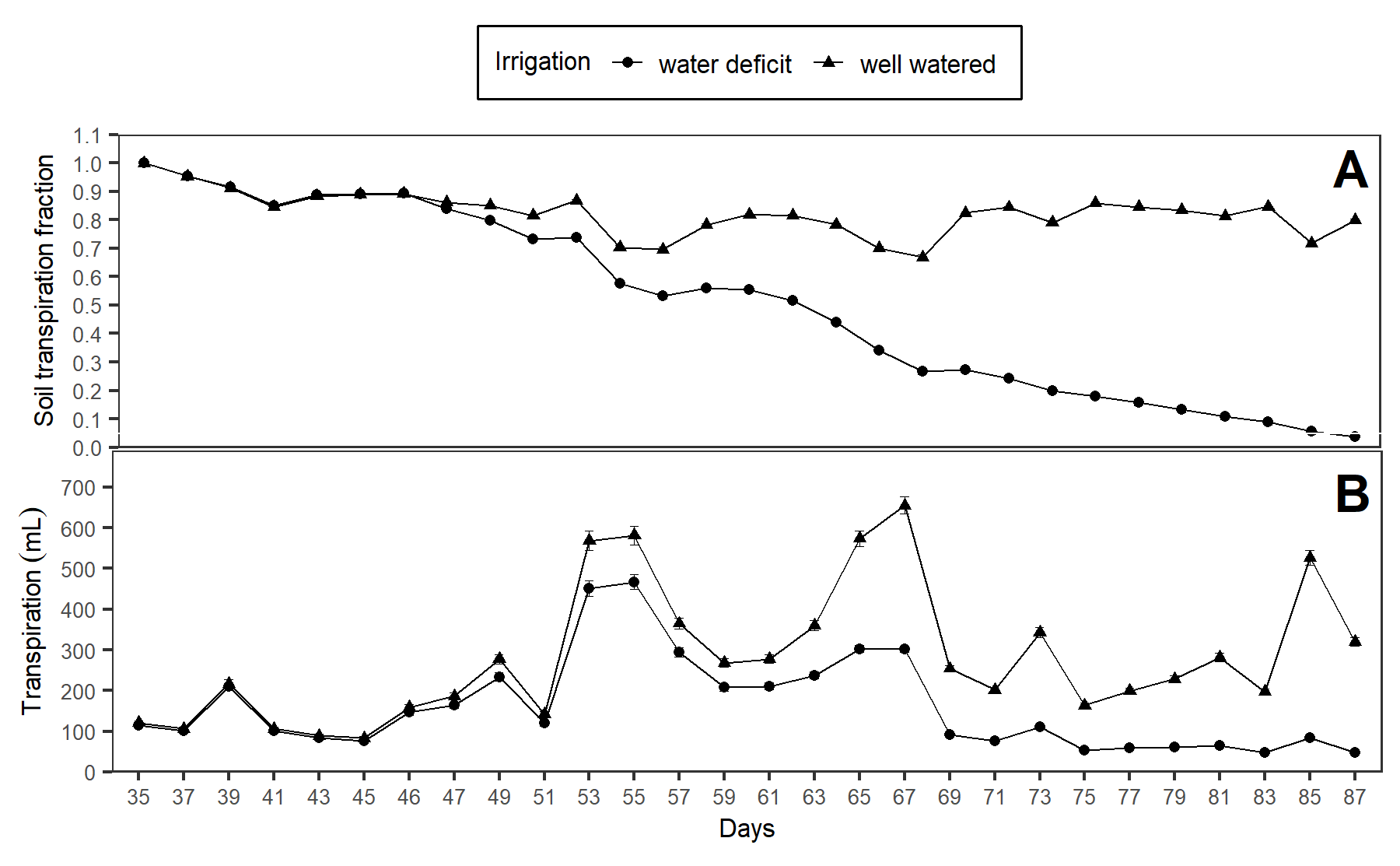
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**Table 1:** List of potato genotypes (*Solanum tuberosum* L.) and their generic characteristics used to assess impact of drought stress. A total of two commercial varieties and 13 genotypes from advanced breeding population developed by the International Potato Center (CIP) were used.

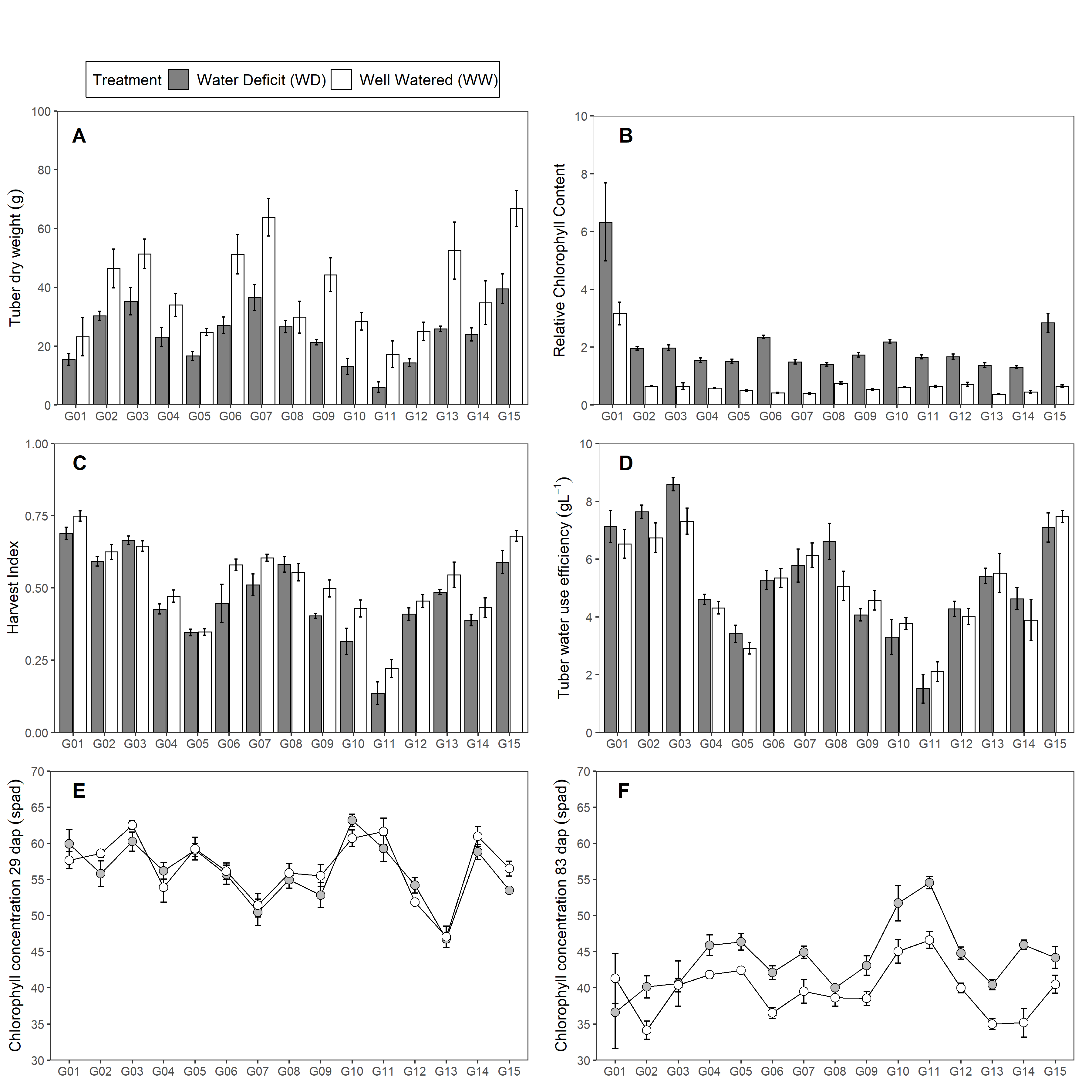
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Number** | **Genotypes** | **Adaptability** | **Growning period** | **Heat tolerance** | **Dry matter (%)** |
| G01 | CIP720088 (Achirana-INTA) |  | early |  | 19 |
| G02 | CIP392797.22 (UNICA) | Lowland and highland Tropics | Medium |  | 21 |
| G03 | CIP397077.16 | Lowland tropics | Medium |  | 20 |
| G04 | CIP398192.213 | Mid elevation tropics | Medium | Tolerant | 22 |
| G05 | CIP398180.612 |  | Medium |  |  |
| G06 | CIP398208.704 | Mid elevation tropics | Medium | Tolerant | 24 |
| G07 | CIP398098.119 | Mid elevation tropics | Medium | Tolerant | 26 |
| G08 | CIP398190.89 | Mid elevation tropics | Medium | Tolerant | 21 |
| G09 | CIP398192.592 | Mid elevation tropics | Medium | Tolerant | 21 |
| G10 | CIP398201.510 | Mid elevation tropics | Medium | Tolerant | 20 |
| G11 | CIP398203.244 | Mid elevation tropics | Medium | Tolerant | 20 |
| G12 | CIP398203.5 | Mid elevation tropics | Medium | Tolerant | 13 |
| G13 | CIP398208.219 | Mid elevation tropics | Medium | Tolerant | 22 |
| G14 | CIP398208.33 | Mid elevation tropics | Medium | Tolerant | 21 |
| G15 | CIP398208.620 | Mid elevation tropics | Medium | Tolerant | 21 |

**Table 2:** Treatment comparison for seventeen variables between Well-Watered (WW) and Water Deficit (WD) in two commercial varieties and 13 genotypes from advanced breeding population developed by the International Potato Center (CIP). Means ± Standard deviation and significance levels (*p*-values) between treatments using T-test is also shown.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **WD**, N = 75 | **WW**, N = 75 | ***p*-value** |
| Chlorophyll Concentration (spad) at 29 DAP | 56.1 ± 4.9 | 56.7 ± 5.0 | 0.4 |
| Chlorophyll Concentration (spad) at 59 DAP | 47.9 ± 4.4 | 45.8 ± 3.7 | 0.002 |
| Chlorophyll Concentration (spad) at 76 DAP | 46.0 ± 5.4 | 41.7 ± 3.6 | <0.001 |
| Chlorophyll Concentration (spad) at 83 DAP | 44.1 ± 5.9 | 39.7 ± 4.5 | <0.001 |
| Plant height (cm) | 132 ± 15 | 150 ± 16 | <0.001 |
| Relative water content (%) | 58 ± 6 | 69 ± 5 | <0.001 |
| Leaf osmotic potential (MPa) | -2.84 ± 0.30 | -2.25 ± 0.29 | <0.001 |
| Leaf dry weight (g) | 12.0 ± 3.7 | 17.3 ± 5.5 | <0.001 |
| Stem dry weight (g) | 11.6 ± 9.1 | 14.5 ± 6.1 | <0.001 |
| Root dry weight (g) | 3.67 ± 1.94 | 3.50 ± 1.96 | 0.6 |
| Tuber dry weight (g) | 24 ± 11 | 40 ± 19 | <0.001 |
| Tuber number (N°) | 12.0 ± 6.2 | 12.0 ± 4.9 | 0.8 |
| Total transpiration (mL) | 4.52 ± 1.22 | 7.85 ± 2.20 | <0.001 |
| Leaf area (cm2) | 2488 ± 797 | 7100 ± 2380 | <0.001 |
| Root length (cm) | 33.1 ± 6.5 | 32.5 ± 5.8 | 0.4 |
| Total dry biomass (g) | 51 ± 16 | 75 ± 24 | <0.001 |
| Harvest Index (hi) | 0.47 ± 0.16 | 0.53 ± 0.14 | 0.020 |
| Specific Leaf Area (cm2g-1) | 218 ± 62 | 415 ± 82 | <0.001 |
| Relative Chlorophyll Content (rcc) | 2.13 ± 1.52 | 0.75 ± 0.73 | <0.001 |
| Biomass water use efficiency (gL-1) | 11.32 ± 2.15 | 9.53 ± 1.26 | <0.001 |
| Tuber Water Use Efficiency (gL-1) | 5.31 ± 2.03 | 5.09 ± 1.75 | 0.5 |



**Figure 1:** (A) Soil transpiration fraction (FTSW) and (B) Daily transpiration in two commercial varieties and 13 genotypes from advanced breeding population developed by the International Potato Center (CIP) under well-watered (WW) and water deficit (WD) condition.



**Figure 2:** Traits measured in two commercial varieties and 13 genotypes from advanced breeding population developed by the International Potato Center (CIP) under well-watered (WW) and water deficit (WD) condition. (A) Tuber dry weight (TDW; g). (B) Relative Chlorophyll Content (RCC). (C) Harvest Index (HI). (D) Tuber Water Use Efficiency (WUEt; gL-1). D-E Chlorophyll Concentration (SPAD). Error bars indicate standard error (n = 5). Whereas, DAP is days after planting.

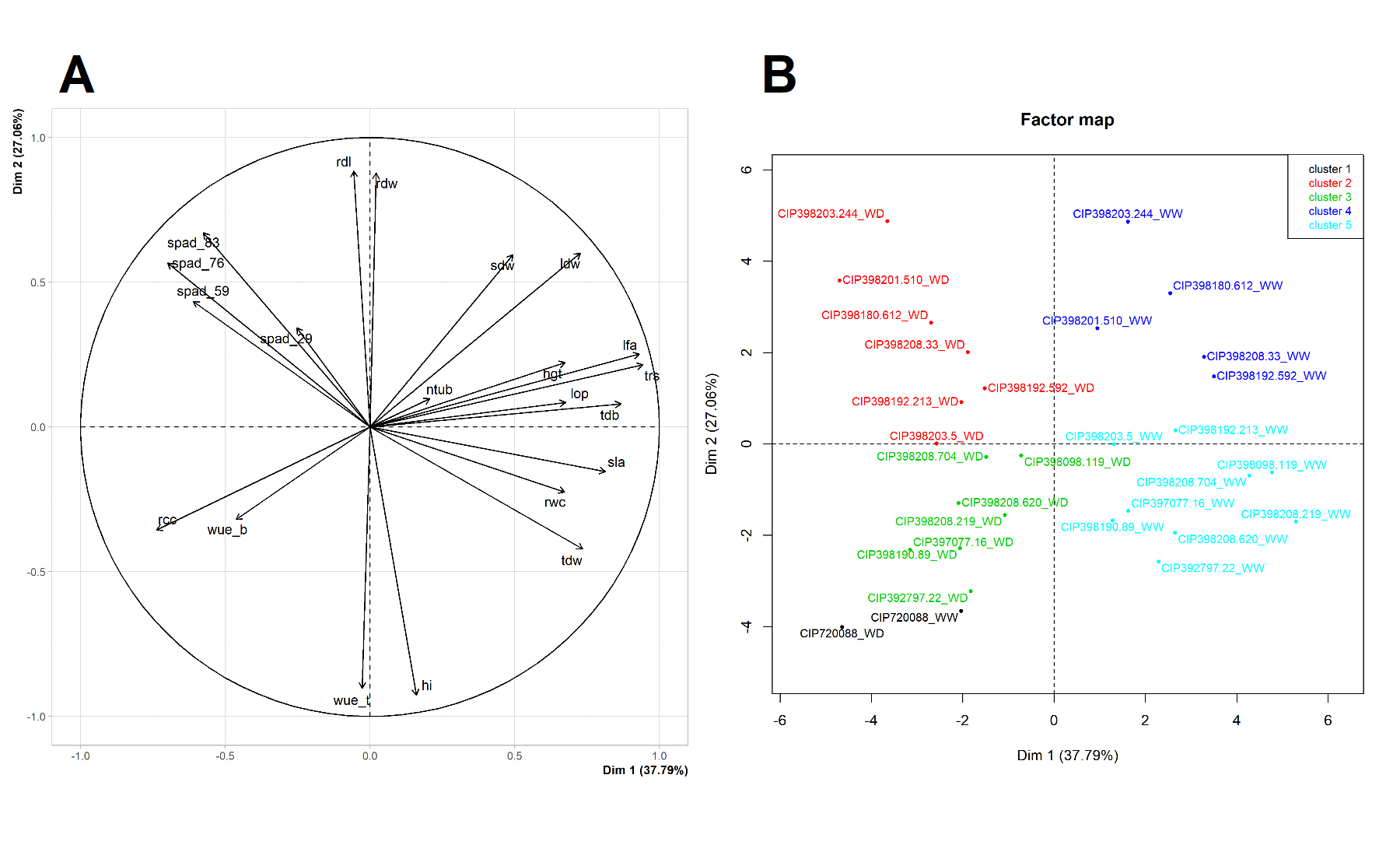


Figure 3: Principal Component Analysis (PCA) for seventeen variables measured in two commercial varieties and 13 genotypes from advanced breeding population developed by the International Potato Center (CIP) under well-watered (WW) and water deficit (WD) condition. (A) PCA for all 17 variables. (B) PCA for 15 genotypes under WW and WD.

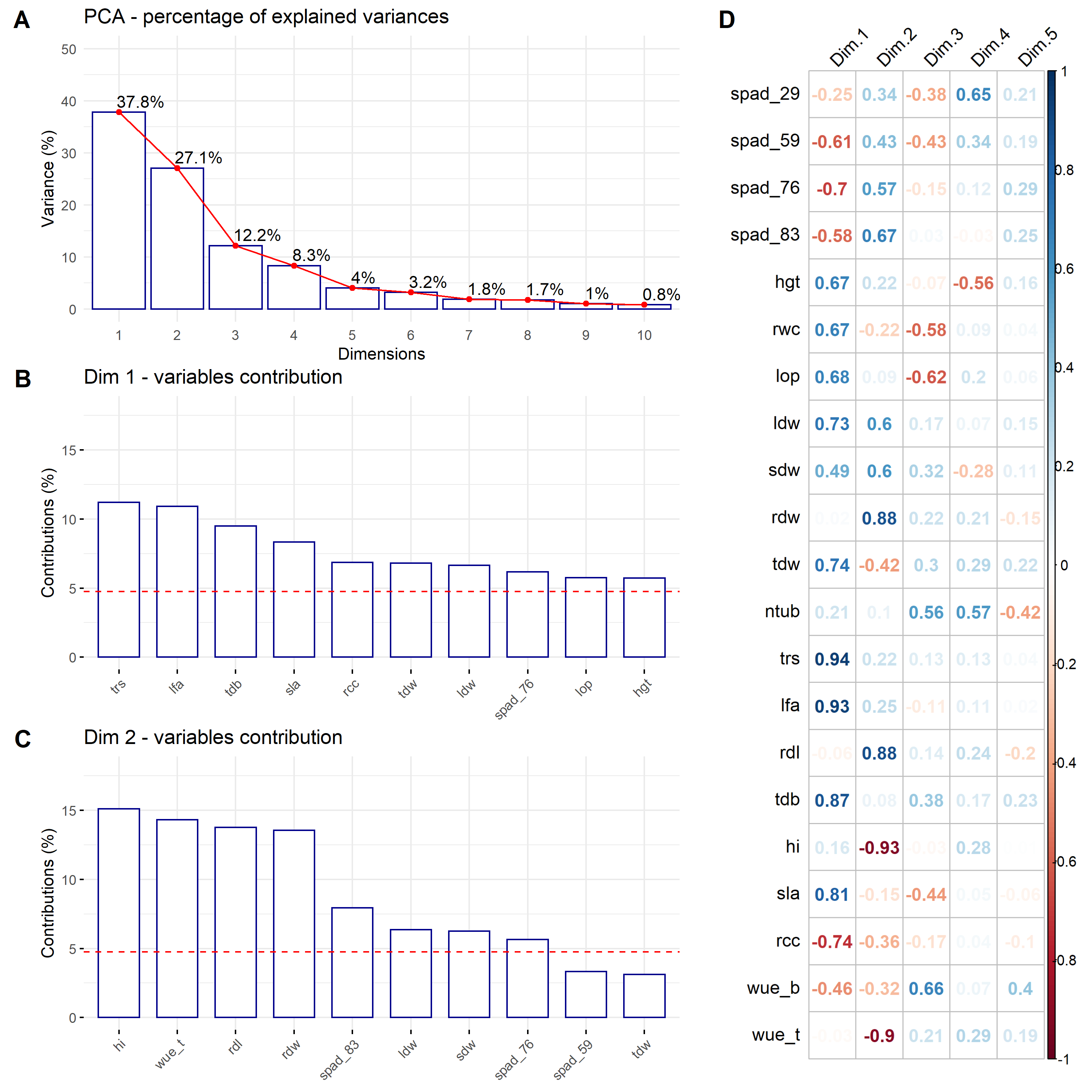
Note: Chlorophyll Concentration (spad), Plant height (hgt; cm), Relative water content (rwc; %), Leaf osmotic potential (lop; MPa), Leaf dry weight (ldw; g), Stem dry weight (sdw; g), Root dry weight (rdw; g), Tuber dry weight (tdw; g), Tuber number (ntub; N°), Total transpiration (trs; mL), Leaf area (lfa; cm2), Root length (rdl; cm), Total dry biomass (tdb; g), Harvest Index (hi), Specific Leaf Area (sla; cm2g-1), Relative Chlorophyll Content (rcc), Biomass water use efficiency (wueb; gL-1), Tuber Water Use Efficiency (wuet; gL-1).

# Supplementary figures



**Figure S1:** Relationships among 17 agro-morphological traits evaluated in well-watered (WW) and water deficit (WD) condition based on Pearson correlation and Euclidean distance measured in two commercial varieties and 13 genotypes from advanced breeding population developed by the International Potato Center (CIP).

**Note:** Chlorophyll Concentration (spad), Plant height (hgt; cm), Relative water content (rwc; %), Leaf osmotic potential (lop; MPa), Leaf dry weight (ldw; g), Stem dry weight (sdw; g), Root dry weight (rdw; g), Tuber dry weight (tdw; g), Tuber number (ntub; N°), Total transpiration (trs; mL), Leaf area (lfa; cm2), Root length (rdl; cm), Total dry biomass (tdb; g), Harvest Index (hi), Specific Leaf Area (sla; cm2g-1), Relative Chlorophyll Content (rcc), Biomass water use efficiency (wueb; gL-1), Tuber Water Use Efficiency (wuet; gL-1).



**Figure S2:** Principal Component Analysis (PCA) among seventeen variables for two commercial varieties and 13 genotypes from advanced breeding population developed by the International Potato Center (CIP). (A) Percentage of the explained variance for each dimension. (B) Variance contribution of the first 10 variables in the dimension 1. (C) Variance contribution of the first 10 variables in the dimension 2. (D) Correlation between the studied variables and among the first 5 dimensions. The reference dashed lines on the bar plot corresponds to the expected value if the contribution between the variables where uniform.



**Figure S3:** Tuber yield (g.pot-1) from five plants of CIP 398203.244 and CIP 398190.89 each, under well-watered (WW) and water deficit (WD) treatments showing impact of treatments. Pictures were taken using the 5 cm scale (black/white segment = 1 cm) displayed alongside the tubers.

# Supplementary Table

**Table S1:** Comparison of 15 potato genotypes under Well-Watered (WW) and Water Deficit (WD) condition. The mean and ± standard desviation values are provided for different traits and indices.

**Note:** Chlorophyll Concentration (spad), Plant height (hgt; cm), Relative water content (rwc; %), Leaf osmotic potential (lop; MPa), Leaf dry weight (ldw; g), Stem dry weight (sdw; g), Root dry weight (rdw; g), Tuber dry weight (tdw; g), Tuber number (ntub; N°), Total transpiration (trs; mL), Leaf area (lfa; cm2), Root length (rdl; cm), Total dry biomass (tdb; g), Harvest Index (hi), Specific Leaf Area (sla; cm2g-1), Relative Chlorophyll Content (rcc), Biomass water use efficiency (wueb; gL-1), Tuber Water Use Efficiency (wuet; gL-1).

**Table S2:** Broad sense heritability in 15 potatos genotypes. Genetic variance (V.g), Error variance (V.e), Standard (h2.s), Cullis (h2.c) and Piepho heritability.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| varible | mean | std | min | max | V.g | V.e | h2.s | h2.c | h2.p |
| spad\_29 | 56.3649 | 3.9152 | 47.4363 | 61.6598 | 16.1978 | 9.1851 | 0.8758 | 0.9463 | 0.9463 |
| spad\_59 | 46.8460 | 3.1094 | 41.2825 | 52.5569 | 10.3681 | 7.5005 | 0.8468 | 0.9322 | 0.9325 |
| spad\_76 | 43.8807 | 3.1786 | 39.5940 | 51.1443 | 11.4327 | 15.0426 | 0.7525 | 0.8837 | 0.8837 |
| spad\_83 | 41.9033 | 3.1628 | 37.8685 | 49.2615 | 11.8096 | 21.3214 | 0.6890 | 0.8468 | 0.8471 |
| hgt | 141.1867 | 12.4217 | 114.0205 | 159.5305 | 169.1887 | 163.2013 | 0.8057 | 0.9120 | 0.9120 |
| rwc | 63.4574 | 0.4351 | 62.6934 | 64.5849 | 1.1869 | 62.5333 | 0.0706 | 0.1595 | 0.1595 |
| lop | -2.5500 | 0.0000 | -2.5500 | -2.5500 | 0.0000 | 0.1734 | 0.0000 |  | 0.0000 |
| ldw | 14.6169 | 3.8339 | 4.3275 | 19.5881 | 15.9965 | 14.1236 | 0.8192 | 0.9189 | 0.9189 |
| sdw | 13.0499 | 4.2903 | 4.4208 | 20.8527 | 21.8256 | 40.5391 | 0.6829 | 0.8399 | 0.8434 |
| rdw | 3.5823 | 1.7621 | 0.8901 | 6.3803 | 3.1811 | 0.7812 | 0.9422 | 0.9751 | 0.9760 |
| tdw | 31.6661 | 10.7894 | 13.9025 | 50.7149 | 131.6490 | 168.5670 | 0.7575 | 0.8750 | 0.8842 |
| ntub | 12.0379 | 2.7755 | 7.1264 | 16.3997 | 9.5482 | 21.8973 | 0.6356 | 0.8038 | 0.8066 |
| trs | 6.1831 | 1.0691 | 3.5631 | 7.8568 | 1.4905 | 4.5330 | 0.5681 | 0.7668 | 0.7668 |
| lfa | 4925.4346 | 952.3186 | 2330.3334 | 6303.8565 | 1431807.6265 | 7188488.7552 | 0.4434 | 0.6336 | 0.6297 |
| rdl | 32.8491 | 4.3555 | 25.2160 | 39.0033 | 20.5711 | 16.6019 | 0.8321 | 0.9102 | 0.9222 |
| tdb | 62.7250 | 13.3849 | 31.5183 | 84.2781 | 209.0848 | 341.6532 | 0.7100 | 0.8476 | 0.8568 |
| hi | 0.4952 | 0.1354 | 0.1859 | 0.7141 | 0.0188 | 0.0044 | 0.9443 | 0.9701 | 0.9764 |
| sla | 320.6928 | 2.7152 | 317.0601 | 326.5109 | 115.9216 | 14981.5968 | 0.0300 | 0.0637 | 0.0622 |
| rcc | 1.3861 | 0.8578 | 0.9228 | 4.3918 | 0.8361 | 0.9819 | 0.7730 | 0.8802 | 0.8792 |
| wue\_b | 10.4248 | 0.5462 | 9.7147 | 11.4392 | 0.5041 | 3.4037 | 0.3720 | 0.5876 | 0.5912 |
| wue\_t | 5.1872 | 1.6600 | 1.8966 | 7.8822 | 2.8288 | 0.7341 | 0.9391 | 0.9638 | 0.9741 |

**Note:** Chlorophyll Concentration (spad), Plant height (hgt; cm), Relative water content (rwc; %), Leaf osmotic potential (lop; MPa), Leaf dry weight (ldw; g), Stem dry weight (sdw; g), Root dry weight (rdw; g), Tuber dry weight (tdw; g), Tuber number (ntub; N°), Total transpiration (trs; mL), Leaf area (lfa; cm2), Root length (rdl; cm), Total dry biomass (tdb; g), Harvest Index (hi), Specific Leaf Area (sla; cm2g-1), Relative Chlorophyll Content (rcc), Biomass water use efficiency (wueb; gL-1), Tuber Water Use Efficiency (wuet; gL-1).