

# Harvest Index a key trait to select tolerant potato genotypes (*Solanum tuberosum*) under drought stress condition

## Abstract

Drought stress, a serious constraint affecting the yields of almost all major crops, is expected to get worse from global climate change and human population expansion. Potato (*Solanum tuberosum* L) is the third most important food crop worldwide and it is highly sensitive to drought stress. To understand the impact of drought stress on yield and to identify traits to select drought-tolerant potato genotypes. We evaluated the response of a range of agronomic and physiological traits in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. The Harvest index (HI) showed high plot-based heritability (0.98) suggesting it can be used for indirect selection for drought-tolerant genotypes. Whereas SPAD was sensitive to detecting early drought stress in potatoes. 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.

**Keywords:** abiotic stress, drought tolerance, physiological traits, plant breeding, SPAD

## Introduction

Drought stress affects agricultural productivity worldwide and is expected to rise in the coming years (Hasegawa et al. 2021). Breeding crops with increased yield potential and improved tolerance to stressful environments is critical for global food security (Raza et al. 2022). The present crop production is insufficient to feed the growing human population by 2050 (Hickey et al. 2019). 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 (Hasegawa et al. 2021). Developing plants with a tolerance to drought relies on various aspects including the drought impact, duration, intensity, crop development stage, and plant genetic potential (Varshney et al. 2021; Seleiman et al. 2021).

Potatoes are the third most consumed food crop worldwide (Devaux et al. 2020), and suffer drastic losses in tuber yield and/or quality due to drought stress (Hill et al. 2021). In general, the potato has a high harvest index in comparison with cereals and a relatively low demand for water i.e., 400 to 600 L for 1 kg of tuber dry matter (Monneveux et al. 2013; Sprenger et al. 2016). Potato cultivars show a variety of responses in morphological, physiological, biochemical, and molecular pathways under drought stress (Aliche et al. 2022). 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 and Ledent 2001; Joshi et al. 2016). 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 (Monneveux et al. 2014). The majority of modern potato cultivars are drought-sensitive presenting different responses to drought stress (Soltys-Kalina et al. 2016; Sprenger et al. 2016). Drought stress tolerance in potatoes is a complex trait controlled by a large number of minor effect quantitative trait loci (QTL). Significant QTLs and differentially expressed genes under drought stress have been identified in potatoes (Khan et al. 2015; Chen et al. 2020).

Water use efficiency (WUE) is an important trait for adaptation to drought stress. And it is defined as the efficiency of the amount of water applied and used for transpiration that goes toward dry matter production. Enhanced WUE can reduce crop water requirements and significantly increase crop yield (Yu et al. 2020). When a higher WUE under drought stress is maintained, the effects of water deficiency are reduced and the competitiveness for water under drought conditions is enhanced (Ogaya and Peñuelas 2003). 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 (Condon 2020).

The identification and select genotypes with high WUE and stable yield under drought stress is a priority to increase genetic gain in potatoes. The main objectives of this work were therefore (1) To understand the drought tolerance mechanisms and physiological responses in 15 potato genotypes under controlled water deficit conditions. (2) To identify traits to select drought-tolerant genotypes with high water use efficiency and low yield penalty.

## Materials and Methods

### *Plant material and experimental conditions*

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 CIP392797.22 (UNICA) with a good yield in warm and dry environments (Gutiérrez-Rosales et al. 2007; Rolando et al. 2015; Demirel et al. 2020); and CIP720088 (Achirana INTA) known for its earliness and drought tolerance (Schafleitner et al. 2007).

The experiment was conducted at the International Potato Center (CIP) experimental station in Lima, Peru (12.1° S, 77.0° W, 244 m.a.s.l.) from May to September 2013. The plants were grown in a controlled greenhouse at 28/15°C average day/night temperature with 70±5% average relative humidity, monitored by a weather station ‘HOBO U12 Outdoor/Industrial model’ (Onset Computer Corporation, Bourne, MA, USA). The potato tubers were pre-sprouted for two weeks in a dark chamber before planting. Afterward, one tuber/genotype was sown at 5–7 cm depth in a 5 L plastic pot containing 5 kg of dry commercial Sogemix SM2 substrate (75% Peat Moss, perlite, vermiculite, and limestone). Fertilization was done twice during the experiment with 10 g of a mixture of granular fertilizer, urea, triple superphosphate, and diammonium phosphate in a ratio of 2:1:2, respectively, one before planting and the other applied at 40 days after planting (dap).

67 Table 1: Potato genotypes (*Solanum tuberosum* L.) used for water deficit experiment with two commercial varieties  
68 and 13 genotypes from an advanced breeding population developed by the International Potato Center (CIP). Source:  
69 International Potato Center (CIP).

Number	Genotypes	Adaptability	Growing 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

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## *Experimental design and irrigation treatments*

The experiment was carried out in a complete randomized block design with two irrigation treatments with five replications of each genotype per treatment. In well-watered (WW) treatment, plants were irrigated according to their transpiration demand (Fig. 1a), and in water deficit (WD) treatment, the water supply was gradually reduced until the wilting point (Ray and Sinclair 1998). At 35 dap, before the stress initiation, the pots were watered to soaking and then allowed to drain overnight (Bhatnagar-Mathur et al. 2007). The next morning, the pots were sealed in a plastic bag secured with a twist tie to prevent water loss except by transpiration and arranged in the greenhouse according to the experimental design. 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 ten days to calculate the initial dry-down parameters for treatment application (Fig. 1b). The WD treatment started at 45 dap which coincides with the beginning of tuber initiation.

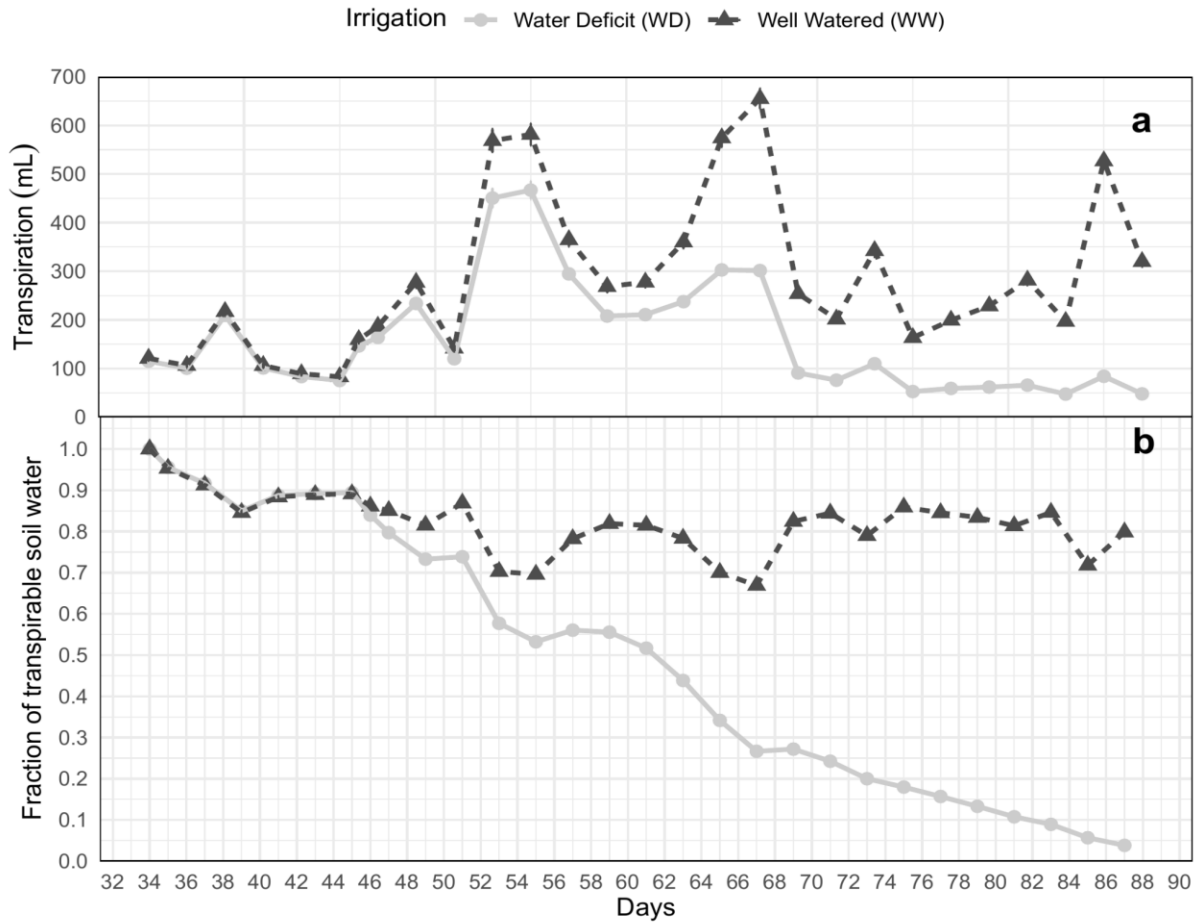


Fig. 1: Water use efficiency in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. (a) Daily transpiration. (b) Fraction of transpirable soil water (FTSW).

## 82 *Transpiration rate*

83 The transpiration rate of each plant was calculated by the procedure previously described by Ray and Sinclair (1998).  
 84 The transpiration rate was calculated by weighing the pots every two days between 13:00 and 15:00 hours (GMT -  
 85 05:00), subtracting the amount of water added, and calculating the difference in weight between the two days. The  
 86 inter-daily transpiration rates of WD plants were normalized against WW plant rates to reduce the influence of day-  
 87 to-day variation, as follows:

$$88 \quad TRS = \left( \frac{WD_{transpiration}}{WW_{transpiration.average}} \right)$$

89 The normalization of the transpiration (TRS) was achieved by dividing the transpiration of each plant in the WD  
 90 regime ( $WD_{transpiration}$ ; mL) by the mean transpiration of the WW plants ( $WW_{transpiration.average}$ ; mL). For comparing the  
 91 transpiration between plants, a second normalization was done so that the normalized transpiration rate (NTR) of each  
 92 plant was defined as 1.0 when the soil water content in each pot was at field capacity (Sinclair and Ludlow 1986). The

available soil water or the fraction of transpirable soil water (FTSW), for each pot, was calculated by dividing the pot weight (g) minus the final pot weight by the transpirable soil water of that pot, using the following formula:

$$FTSW = \left( \frac{pot.weight_{inter-daily} - pot.weight_{final}}{pot.weight_{initial} - pot.weight_{final}} \right)$$

The inter-diary transpiration rate for each pot on average was 275.7 mL for WW and 72.5 mL for WD plants (Fig. 1a). The FTSW in WW plants was maintained above 70%, while for the WD treatment, the gradual restriction in water supply decreased the water availability. The effect of the FTSW under WD was visible after 8 days of water restriction was applied (Fig. 1b). The plants were harvested when the plants in WD had less than 10% of FTSW (Fig. 1b).

### *Agro-physiological traits*

**Water Use Efficiency (WUE; g/L):** We have calculated the biomass water use efficiency ( $WUE_B$ ) and tuber water use efficiency ( $WUE_T$ ). The  $WUE_B$  was calculated as the total biomass in dry weight (g) produced divided by the cumulative water transpired; for  $WUE_T$  we used the dry weight (g) from tuber production divided by the total water transpired (TRS; L) during the irrigation treatment.

**Relative Water content (RWC; %):** Relative water content was determined by weighing the fresh weight (leaflet<sub>FW</sub>; g) of the 3rd leaflet from the youngest fully expanded leaf in the third leaf from the apical part for each plant 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 before taking turgid weight (leaflet<sub>TW</sub>; g). Leaves were reweighed after drying (leaflet<sub>DW</sub>; g) in an oven overnight at 90°C. All the components were weighted on a precision scale (0.001 g). RWC was calculated following the formula described by Vasquez-Robinet et al. (2008):

$$RWC(\%) = \left( \frac{leaflet_{FW} - leaflet_{DW}}{leaflet_{TW} - leaflet_{DW}} \right) \cdot 100$$

**Leaf Osmotic Potential (LOP; MPa):** Leaf Osmotic Potential was determined using a dew point microvoltmeter (HR-33T Wescor Inc., Logan, UT, USA) with leaf discs of 5 mm diameter, taken from the third fully extended leaf. The leaf discs were put in 1 mL cryogenic tubes and frozen in liquid nitrogen. The frozen leaves were incubated at 22°C for 30 min in a sealed C-52 chamber (Wescor Inc., Logan, UT, USA).

**Relative chlorophyll content (SPAD):** Relative chlorophyll content of leaves was evaluated by taking SPAD (Soil Plant Analysis Development) measurements using a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc., Osaka, Japan) from the youngest fully expanded leaf in the third leaf from the apical part in three different points on the leaflet. Individual readings of leaflets were averaged to represent the individual measurement of a leaf. The SPAD was taken throughout plant development at 29, 59, 76, and 83 dap. At 29 dap all plants were before the stress was imposed.

**Morphological traits:** The harvest was performed at 90 dap when the plants in WD had less than 10% of FTSW. The plant height (HGT, cm) was recorded from the base of the soil to the top of each plant with a measuring tape. After that, each plant was cut to the soil level and washed to remove all substrate. The tuber number (NTUB) and the root

length (RTL, cm) were recorded. In the case of TDW, all the tubers were chopped before being set in the oven. Each plant was separated into four components: leaves, stems, roots, and tubers. The leaf area (LFA; cm<sup>2</sup>) was measured with the fresh leaves. The leaves were arranged on a wooden board and they were photographed. Each picture was analyzed using ImageJ software (Zárate-Salazar et al. 2018).

**Post-harvest evaluations:** The dry weight of leaves (LDW; g), stems (SDW; g), roots (RDW; g), and tubers (TDW; g) was determined with a precision scale (0.01 g) after drying all the components individually in kraft bag paper at 80°C for three days in a forced-air oven. The total dry biomass (TDB; g) was calculated with the sum of all components.

**Indices:** The harvest index (HI) was calculated as the ratio of TDW related to the TDB. The specific leaf area (SLA; cm<sup>2</sup>/g) was calculated by dividing LFA by LDW. Relative chlorophyll content (RCC) was calculated between the relation of SPAD at 83 dap and LFA.

### *Statistical analysis*

The phenotype  $P_{ij}$  for genotype  $i$  in the block  $j$  was modeled by:  $P_{ij} = \mu + G_i + B_j + \epsilon_{ij}$  according to Zystro et al. (2018). Where  $\mu$  is the intercept and  $G_i$ ,  $B_j$  and  $\epsilon_{ij}$  are normally distributed random effects for genotype, block, and residuals, respectively. Variance components were estimated by restricted maximum likelihood implemented in the H2cal() function implemented in the *inti* package (Lozano-Isla 2023). Plot-based heritability was estimated by:  $H^2 = \frac{\theta_G^2}{\theta_G^2 + \theta_\epsilon^2}$ . Where  $\theta_G^2$  and  $\theta_\epsilon^2$  are the variance components for genotype and residual, respectively.

The statistical analysis was performed using R version 4.2.2 (R Core Team, 2020). A Student's t-test was performed between WW and WD treatment (p<0.05) with the *gtsummary* package (Sjoberg et al. 2023). The Student-Newman-Keuls mean comparison test (p<0.05) was performed to evaluate the differences between the treatments and genotypes implemented in the *agricole* package (Mendiburu 2021). The principal components analysis (PCA) and graphics were used in the *FactoMineR* package (Lê et al. 2008; Kassambara and Mundt 2020). The correlation graphic was performed with *psych* (Revelle 2022) and *corrplot* packages (Wei et al. 2021).

The code, dataset, and reproducible data analysis were performed under Quarto (Allaire et al. 2023) an open-source scientific and technical publishing system (Supplementary File 1).



## Results

### *Agronomic and physiological traits response to water deficit*

The water deficit during the tuber initiation can reduce the yield and the quality of potatoes. To compare the response and the impact of the drought stress in potatoes, 15 genotypes were exposed to water deficit conditions at the beginning of tuber initiation (i.e 45 dap). Eighteen (18) traits among agronomic and physiological traits were evaluated during the water deficit stress-controlled condition.

The plants did not show differences in SPAD at 29 dap (Figure 2a); while SPAD at 83 dap showed differences between treatment and genotypes (Figure 2b). The SPAD values were lower at 83 than at 29 dap (Figure 2a-b, Table 2). The SPAD in WD treatment for all the genotypes was higher than the ones at WW conditions (Table 2, Figure 2b). The genotypes CIP398190.89 and CIP720088 had the lowest differences for SPAD at 83 dap among treatments with 2.06% and 0.30% respectively, while CIP398203.244 and CIP398208.33 had the largest values with 14.48 and 17.54%, respectively (Figure 2b).

All the genotypes decreased the RWC and LOP in response to WD (Table 2) with values ranging between 64.96% and 50.09% for CIP720088 and CIP398201.510, respectively. The SLA reduction was 48% under WD treatment compared to WW (Table 2). The lowest reduction for SLA was among the genotypes CIP398190.89 and CIP398203.5 with 24%, and 21% respectively; while the highest SLA reduction where found for CIP398208.219, CIP398098.119, and CIP398208.704 with 53%, 65%, and 64% respectively.

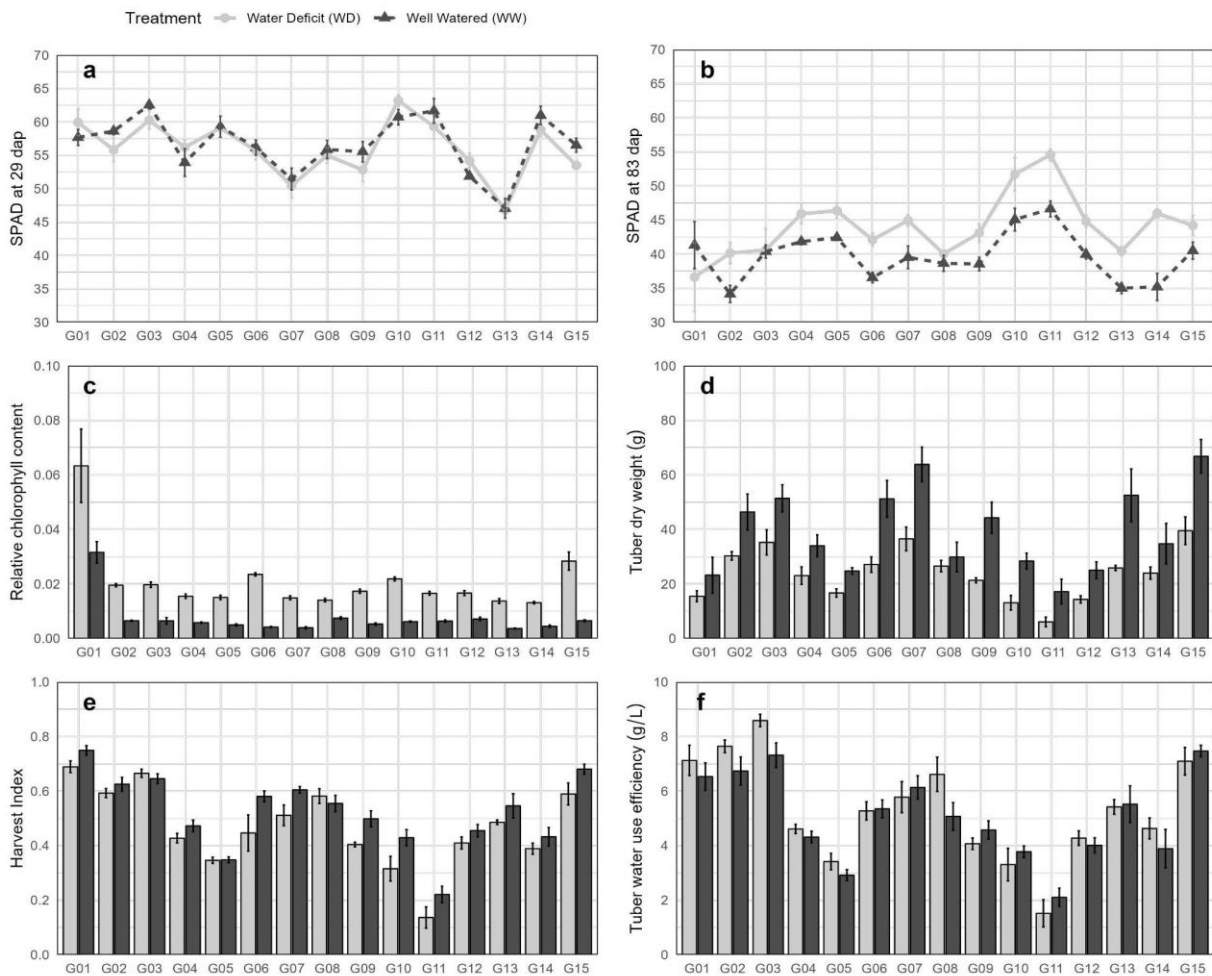


Fig. 2: Traits measured in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. (a-b) Chlorophyll concentration. (c) Tuber dry weight. (d) Relative chlorophyll content. (e) Harvest Index. (f) Tuber Water Use Efficiency. Error bars indicate standard error (n = 5). Days after planting (dap).

The morphological traits HGT, LDW, SDW, and LFA decreased significantly under WD (Table 2). There was a drastic reduction of 65% in plants under WD for LFA compared to WW plants (Table 2). We did not find differences between the irrigation treatment for NTUB, RDW, and RTL (Table 2). The RCC allowed discriminating genotypes between WD and WW (Figure 2c). The genotypes with best performance for RCC were CIP720088 (Achirana-INTA), CIP398208.620, CIP398208.704, CIP398201.510, CIP392797.22 (UNICA) and CIP397077.16 (Figure 2c).

175 Table 2: Response comparison for 18 traits in 15 potato genotypes under Well-Watered (WW) and Water Deficit  
176 (WD) conditions. The values for different traits are represented by the mean  $\pm$  standard deviation with the significance  
177 under the t-test with their respective p-values.

Variable	Water deficit	Well-Watered	p-value
Chlorophyll concentration (SPAD) at 29 dap	56.1 $\pm$ 4.9	56.7 $\pm$ 5.0	0.4
Chlorophyll concentration (SPAD) at 59 dap	47.9 $\pm$ 4.4	45.8 $\pm$ 3.7	0.002
Chlorophyll concentration (SPAD) at 76 dap	46.0 $\pm$ 5.4	41.7 $\pm$ 3.6	<0.001
Chlorophyll concentration (SPAD) at 83 dap	44.1 $\pm$ 5.9	39.7 $\pm$ 4.5	<0.001
Plant height (cm)	132 $\pm$ 15	150 $\pm$ 16	<0.001
Relative water content (%)	58 $\pm$ 6	69 $\pm$ 5	<0.001
Leaf osmotic potential (MPa)	-2.84 $\pm$ 0.30	-2.25 $\pm$ 0.29	<0.001
Leaf dry weight (g)	12.0 $\pm$ 3.7	17.3 $\pm$ 5.5	<0.001
Stem dry weight (g)	11.6 $\pm$ 9.1	14.5 $\pm$ 6.1	<0.001
Root dry weight (g)	3.67 $\pm$ 1.94	3.50 $\pm$ 1.96	0.6
Tuber dry weight (g)	24 $\pm$ 11	40 $\pm$ 19	<0.001
Tuber number (N°)	12.0 $\pm$ 6.2	12.0 $\pm$ 4.9	0.8
Total transpiration (mL)	4.52 $\pm$ 1.22	7.85 $\pm$ 2.20	<0.001
Leaf area (cm <sup>2</sup> )	2,488 $\pm$ 797	7,100 $\pm$ 2,380	<0.001
Root length (cm)	33.1 $\pm$ 6.5	32.5 $\pm$ 5.8	0.4
Total dry biomass (g)	51 $\pm$ 16	75 $\pm$ 24	<0.001
Harvest index (HI)	0.47 $\pm$ 0.16	0.53 $\pm$ 0.14	0.020
Specific leaf area (cm <sup>2</sup> g <sup>-1</sup> )	218 $\pm$ 62	415 $\pm$ 82	<0.001
Relative chlorophyll content (RCC)	0.021 $\pm$ 0.015	0.008 $\pm$ 0.007	<0.001
Biomass water use efficiency (g L <sup>-1</sup> )	11.32 $\pm$ 2.15	9.53 $\pm$ 1.26	<0.001
Tuber water use efficiency (g L <sup>-1</sup> )	5.31 $\pm$ 2.03	5.09 $\pm$ 1.75	0.5

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The TDB for WD reduced ca. 32% in comparison with the WW treatment (Table 2, Fig. 3). In terms of productivity, WD treatment decreased TDW across genotypes by ca. 40% (Figure 2d). The genotype CIP398190.89 had greater TDW in WD treatment compared to its yield in WW treatment with a 5% increase in biomass, while other genotypes like CIP398203.5 and CIP398203.244 presented up to 56% and 48% reduced tuber production (Figure 2d). The genotypes CIP398203.244, CIP398180.612, and CIP398201.510 were among the most sensitive genotypes at 31.6%, 46.7%, and 48.9% respectively under WD (Figure 2d).

Significant differences were found for HI among genotypes and treatments (Figure 2e, Table 2). Genotypes showed HI at ca. 53% under WW conditions and the water deficit was reduced by 11% (Table 2).  $WUE_B$  was generally higher for genotypes under WD treatment than under WW treatment (Table 2). Under WD treatment, CIP397077.16, CIP398208.620, CIP392797.22, and CIP398190.89 showed the highest  $WUE_B$  with 13.06, 12.03, and 11.59 g/L, respectively. The lowest  $WUE_B$  with 8.50 and 9.24 g/L were presented by CIP398180.612 and CIP398203.5, respectively. For  $WUE_T$  there is no significant difference between treatments (Table 2). The genotypes with higher  $WUE_T$  under WD treatment were CIP397077.16, CIP392797.22 (UNICA), CIP720088, and CIP398208.620 (Figure 2f).

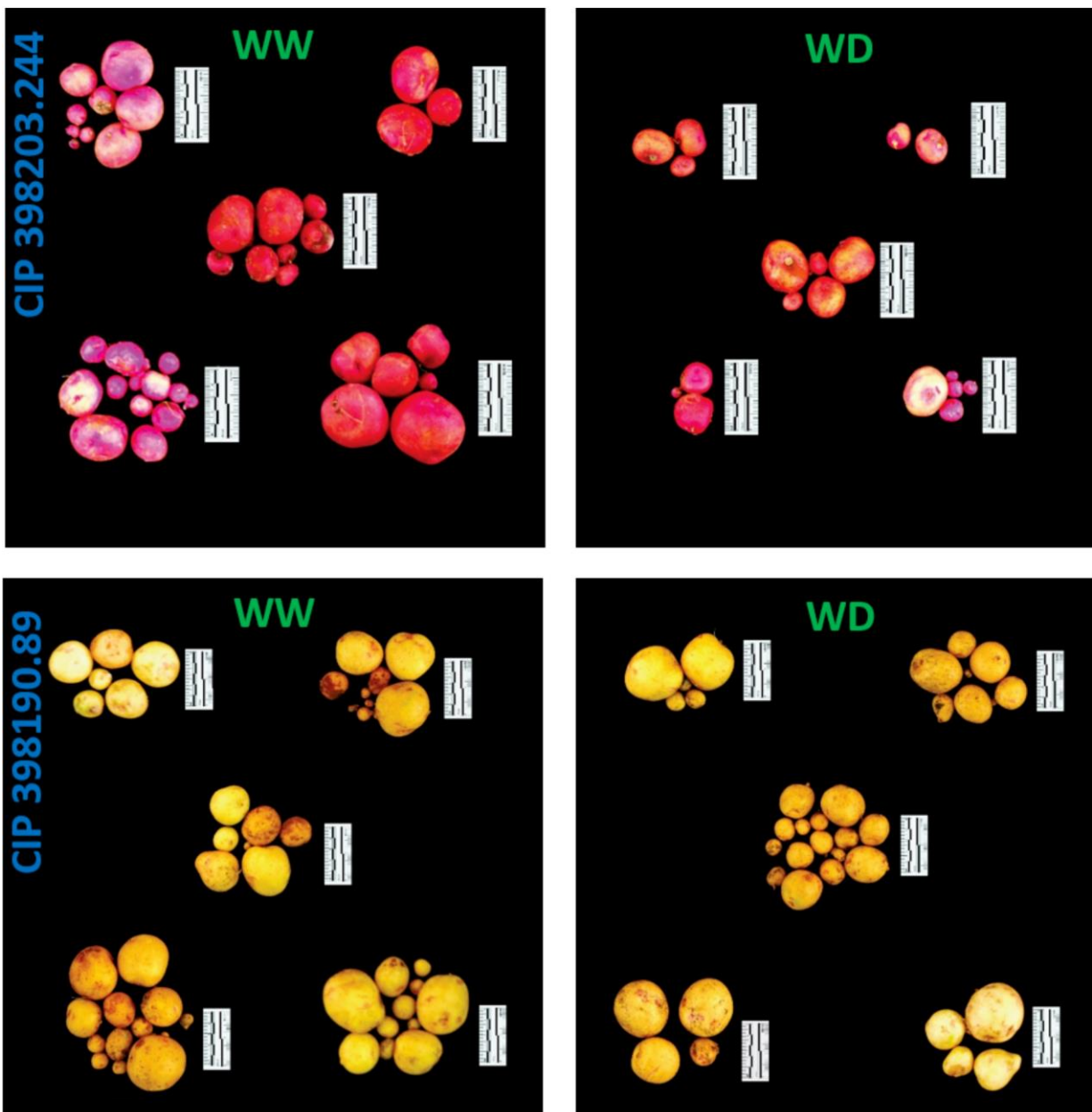


Fig. 3: Tuber yield performance in two contrasting potato genotypes under well-watered (WW) and water deficit (WD) conditions. CIP 398203.244, susceptible genotype shows good yield performance under well-watered (WW) but reduced performance under water deficit (WD) conditions. CIP 398190.89, tolerance genotype with similar yield response under water deficit (WD) and well-watered (WW). Each group represents one replication ( $n = 5$ ). Pictures were taken using the 5 cm scale (black/white segment = 1 cm) displayed alongside the tubers.

## *Genotype selection under water deficit*

Selection of genotypes with enhanced yield performance under field conditions is time-consuming and laborious, especially for large populations as required in breeding programs and genetic analysis. Correlation, PCA, and plot-based heritability were performed to identify useful traits for the indirect selection in potato genotypes under water deficit.

The TDW had a positive correlation with TDB ( $r = 0.84$ ), HI ( $r = 0.61$ ), and TRN ( $r = 0.71$ ) and a consistent negative correlation in the three measurements of SPAD at 83 dap ( $r = -0.60$ ) (Fig. 4). LFA showed a high correlation with TRS ( $r = 0.93$ ) and LDW ( $r = 0.85$ ) while presenting a negative correlation with RCC ( $r = -0.76$ ) (Fig. 4). The HI presents a negative correlation with SPAD at 83 dap ( $r = -0.7$ ), RDW ( $r = -0.73$ ), and RTL ( $r = -0.75$ ); while  $WUE_T$  showed a strong positive correlation with HI ( $r = 0.92$ ) and negative correlation with RTL ( $r = -0.73$ ). The RCC presented a negative correlation with TRS ( $r = -0.76$ ), LFA ( $r = -0.76$ ), and LDW ( $r = -0.78$ ) (Fig. 4).

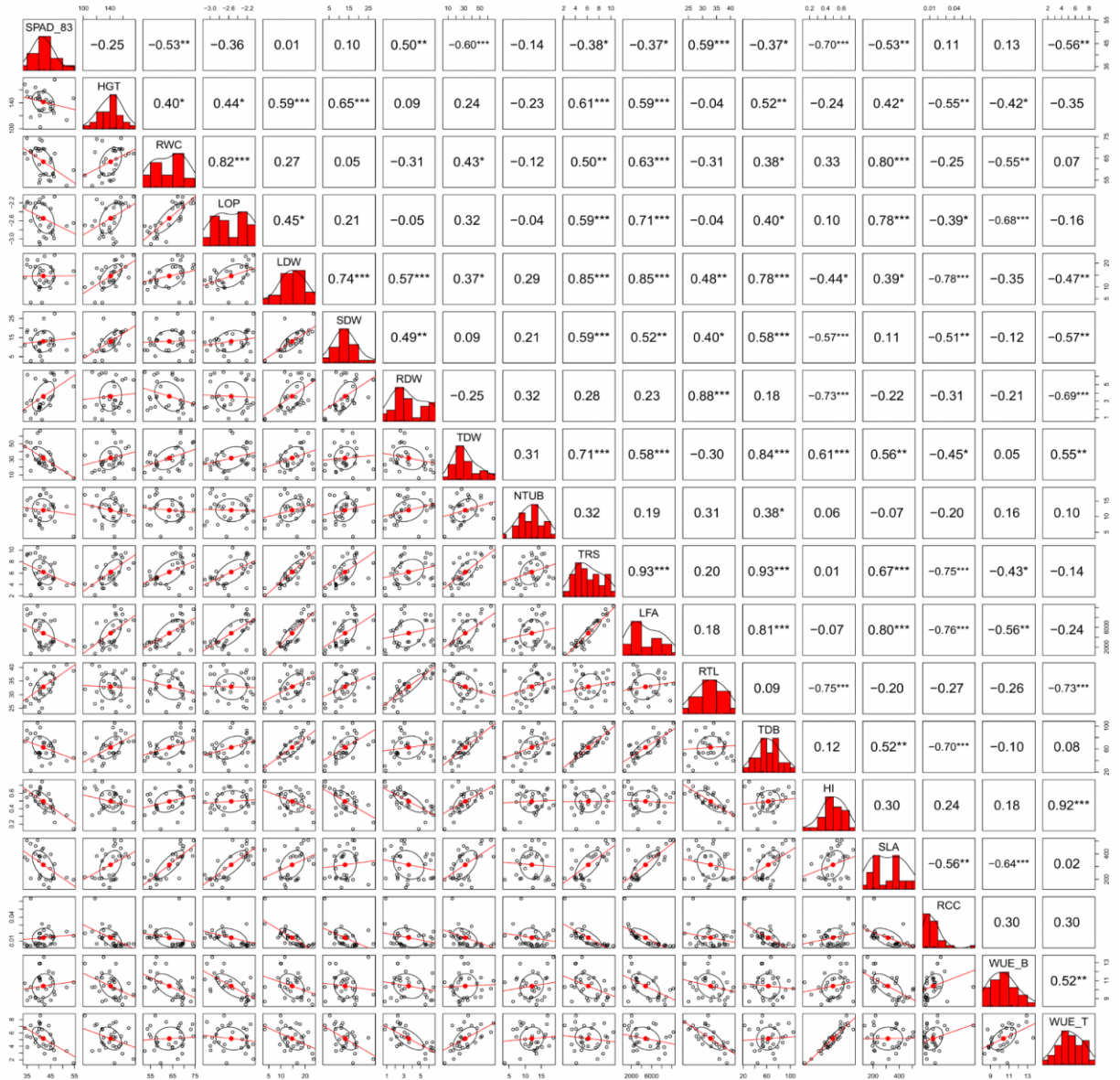


Fig. 4: Pearson correlation among agronomic and physiological traits evaluated in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. Correlation coefficients (above diagonal), linear regressions (below diagonal), and trait distribution (diagonal in red color). p-value: 0.05\*, 0.01\*\*, 0.001\*\*\*. Where: 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; cm<sup>2</sup>), Root length (RTL; cm), Total dry biomass (TDB; g), Harvest index (HI), Specific leaf area (SLA; cm<sup>2</sup> g<sup>-1</sup>), Relative chlorophyll content (RCC), Biomass water use efficiency (WUE<sub>B</sub>; g L<sup>-1</sup>), Tuber water use efficiency (WUE<sub>T</sub>; g L<sup>-1</sup>).

To understand the interaction and response of the genotypes under WW and WD conditions a PCA for the traits and individuals was performed. The first two components in the PCA explained 64.9% of the variance (Fig. 5, Fig S1a). In the first dimension, the five traits with the highest contribution were TRS, LFA, TBD, SLA, and RCC (Fig. S1b). In the second dimension, the traits with major contributions were HI, WUE<sub>T</sub>, RTL, RDW, and SPAD 83 dap (Fig.

S1c). In the first dimension, there was a positive correlation between LDW, LFA, and TRS with a negative correlation with RCC and WUE<sub>B</sub> (Fig. 5a, Fig. S1d). The genotypes with high LFA presented more TRS and LDW but they have low RCC and WUE<sub>B</sub> (Fig. 5a, Fig. S1d). In the second dimension, RTL and RWD were correlated and presented negative correlations with HI and WUE<sub>T</sub> (Fig. 5a, Fig. S1d). The plot-based heritability presented high values for HI (0.96), RDW (0.95), WUE<sub>T</sub> (0.94), SDW (0.89), and RTL (0.83). And, low values for RWC (0.07), LFA (0.44), SLA (0.03), and OP (<0.001) (Fig. 5a, Table S1)

The PCA for the individuals grouped the genotypes in five clusters (Fig. 5b). Cluster 1 was associated with the genotype Achirana-INTA with early maturity (Table 1). Clusters 2 and 3 are associated with the genotypes under WD conditions; while clusters 4 and 5 are related to the genotypes under WW conditions. In the distribution between the individuals and traits, the genotypes UNICA, CIP397077.16, CIP398190.89, and CIP398208.620 are located in clusters 3 and 5 presented higher performance under WD conditions with high WUET, HI, and RCC (Fig. 5, Figure 2c, e-f). The genotypes CIP398203.244, CIP398180.612, CIP398201.510, and CIP398192.592 located in clusters 2 and 4 presented low performance under both treatments (Fig. 5b, Fig. S2).





## Discussion

In the present research, we studied two commercial varieties and thirteen potato genotypes from the advanced breeding population from the International Potato Center under a water deficit in controlled greenhouse conditions. The evaluated genotypes triggered a range of physiological, morphological, and survival strategies mechanisms to lead to the water deficit condition. Evaluating WUE under field conditions can be tedious and expensive due to the difficulty in accurately measuring water consumption in the plants. Therefore, traits that are easier to evaluate in a large number of genotypes are required. We found that Harvest Index (HI) and SPAD are key indicators for tuber water use efficiency ( $WUE_T$ ) in potatoes. These two traits can be scored easily under field conditions and be useful in the selection of drought-tolerant genotypes with high water use efficiency with stable yields. Additionally, we identified the high performed genotypes under water deficit conditions.

Plants develop drought tolerance strategies through improved morphological, physiological, biochemical, and cellular mechanisms (Raza et al. 2022). Our results showed that genotypes with the longest RTL and high LFA as CIP398201.510 and CIP398203.244 have the lowest yield under both water-stressed and non-stressed conditions. These genotypes preferred to increase shoot biomass, leaves and stems over tuber production. The long roots also seem to contribute to vegetative growth rather than tuber production, similar to results found by Zarzyńska et al. (2017) under greenhouse conditions showing a strong correlation between root length and yield decrease in five potato cultivars.

The reduction of transpiration due to the reduction of leaf area is another drought-resistance mechanism presented in potatoes. Thick leaves often have greater photosynthetic capacity than thin leaves, due to an increase in chlorophyll content per leaf area (Songsri et al. 2009; Rolando et al. 2015; Aliche et al. 2020). We found that some genotypes have reduced their transpiration and maintained high SPAD values to allow increased  $WUE_T$  under water deficit conditions. Similar results were also reported by Rodríguez-Pérez et al. (2017) in Andean potato genotypes. This response is also reflected in our work by the maintenance of high RCC in the drought-stressed genotypes. The inclusion of RCC allows differentiating the genotypes with higher yield performance under WD conditions (Fig. 4, Figure 2c-d). Apparently, genotypes with these characteristics allocate a higher fraction of assimilates to tubers by reducing the evaporative surface area above ground and efficiently utilizing the water and N resources in the soil (Satognon et al. 2022). Our results suggest SPAD measures were sensitive to detect drought stress even in the early stages of the stress in potatoes (Table 2).

Agronomically, maintaining high yield performance under drought stress is a crucial mechanism in potatoes (Boguszewska-Mańkowska et al. 2018). Under well-watered conditions, soil can supply water at a steady rate to meet transpiration demand. However, as the soil becomes dry, water flux from the soil to the root surface decreases and cannot satisfy the demand for transpiration (Aliche et al. 2020). We found a significant and strong positive correlation between HI and  $WUE_T$ , indicating a direct association between these two traits. We found minimal yield loss in genotypes like CIP720088, CIP392797.22 (UNICA), CIP397077.16, and CIP398190.89 under water stress. This response was related to the ability of these genotypes to increase their  $WUE_T$  by absorbing the limited water and nutrients available in the soil without decreasing yield (Kaminski et al. 2015; Reddy et al. 2020). This suggests the

relationship between biomass production,  $WUE_T$ , and HI when limited soil water is available. HI is a stable trait for particular cultivars over a wide range of conditions (Donald and Hamblin, 1976; Khan et al., 2015). In the present study, the HI presented a high plot-based heritability. Suggesting that selecting genotypes with high HI under water-limited conditions is especially important to obtain individuals with high  $WUE_T$  with high yield performance.

The main limitation in the present work was the limited space in the greenhouse to include additional pots to adjust the water requirement in time for each treatment. However, the difference between the water deficit treatment and stress condition was achieved (Fig. 1) as all the pots have the same source of variation and the error were distributed normally.

In conclusion, tolerant genotypes preferentially use available water-increasing to increase their tuber production rather than above-ground biomass whereas susceptible genotypes presented high transpiration and long RTL with low  $WUE_T$ . The SPAD was sensitive to detect early drought stress in potatoes. The HI was related to  $WUE_T$  and yield performance under water deficit conditions and its present high heritability. Our results suggest HI as an efficient trait to select drought stress tolerance genotypes and could be useful as selection criteria in breeding programs where it is required to evaluate a large number of genotypes under field conditions.

#### **Data availability**

The dataset, code, and reproducible data analysis are presented in Supplementary File 1.

#### **Conflict of interest**

Authors declares no conflicts of interest.

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