1   **Harvest index is a key trait for screening drought-tolerant potato genotypes (*Solanum tuberosum*)**

2

3   **Abstract**

4   Drought stress is a significant constraint that affects the yield of almost all major crops. Its impact is expected to

5   worsen due to global climate change and human population expansion. Potato (*Solanum tuberosum* L) is the third

6   most important food crop worldwide, but it is highly susceptible to drought stress. In order to understand the effects

7   of drought stress on yield and identify traits for selecting drought-tolerant potato genotypes, we evaluated the response

8   of a range of agronomic and physiological traits in 15 potato genotypes under well-watered (WW) and water deficit

9   (WD) conditions. The harvest index (HI) was found to have a high plot-based heritability (0.98), indicating that it can

10   be used for the indirect selection of drought-tolerant genotypes. In contrast, SPAD was sensitive to detecting early

11   drought stress in potatoes. Drought-tolerant genotypes, including CIP392797.22 (UNICA), CIP397077.16,

12   CIP398190.89, CIP398208.219, and CIP398208.620, were able to allocate limited water towards tuber production

13   rather than biomass. These genotypes showed high tuber production under WW conditions and increased

14   photosynthetic activity and water use efficiency under WD conditions.

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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). However, 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).

Potatoes show a variety of responses in morphological, physiological, biochemical, and molecular pathways under drought stress (Aliche et al. 2022). Water deficit induced by drought is a significant abiotic stress factor that has detrimental effects on potato growth, development, and tuber yield (Soltys-Kalina et al. 2016; Sprenger et al. 2016). To address water scarcity, potato plants have developed various physiological, biochemical, and molecular mechanisms. These mechanisms enable them to optimize water use efficiency, maintain cellular homeostasis, and minimize oxidative damage. Potato plants display several physiological responses aimed at mitigating the negative impacts of drought. These responses include stomatal closure, which reduces transpirational water loss, and changes in leaf morphology, such as a decrease in leaf area and an increase in leaf thickness, to limit water vapor diffusion (Deblonde and Ledent 2001; Joshi et al. 2016). Additionally, potatoes undergo adjustments in their root system architecture, promoting deeper and more extensive root growth to access water from deeper soil layers (Khan et al. 2016). In terms of molecular and biochemical mechanisms, they involve the accumulation of osmoprotectants, such as proline, sugars, and compatible solutes (Obidiegwu et al. 2015). These substances help maintain cellular osmotic potential and protect macromolecules from damage caused by dehydration. Antioxidant defense systems are also upregulated, including the synthesis of enzymes like superoxide dismutase, catalase, and peroxidase, which scavenge reactive oxygen species and prevent oxidative stress (Lal et al. 2022). Furthermore, drought-tolerant potato varieties exhibit enhanced expression of stress-responsive genes involved in signal transduction, transcriptional regulation, and stress tolerance pathways (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

54   drought conditions is enhanced (Ogaya and Peñuelas 2003). Cultivars can be bred for WUE, increasing yield per unit

55   of water, by capturing traits that help the plants develop faster, flower earlier, have a lower leaf area index, and be

56   more efficient in capturing radiation (Condon 2020).

57   The identification and selection of genotypes with high water use efficiency (WUE) and stable yield under drought

58   stress are crucial steps toward achieving greater genetic gain in potatoes. The main objectives of this work were: (1)

59   To investigate the drought tolerance mechanisms and physiological responses of potatoes under water deficit

60   conditions, and (2) To identify traits associated with drought tolerance and high WUE that would enable the selection

61   of genotypes capable of maintaining productivity even under limited water availability.

62

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 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 performed twice during the experiment. The initial application occurred prior to planting, whereby the fertilizers were mixture with the substrate. The second application was administered at the surface and incorporated 40 days after planting (dap). The substrate fertilizer application rates were 7-1-4 de N-P-K consisted of 51.8 kg/ha of N, 33.8 kg/ha of P_2O_5, and 75 kg/ha of K_2O. Nitrogen was applied in the form of ammonium nitrate (34.5% N), phosphorus was provided as triple superphosphate (45% P_2O_5), and potassium was delivered using potassium sulphate (50% K_2O).

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

Experimental design and irrigation treatments

87 The experiment was carried out in a complete randomized block design with two irrigation treatments with five

88 replications of each genotype per treatment. In well-watered (WW) treatment, plants were irrigated according to their

89 transpiration demand (Fig. 1a), and in water deficit (WD) treatment, the water supply was gradually reduced until the

90 wilting point (Ray and Sinclair 1998). At 35 dap, before the stress initiation, the pots were watered to soaking and

91 then allowed to drain overnight (Bhatnagar-Mathur et al. 2007). The next morning, the pots were sealed in a plastic

92 bag secured with a twist tie to prevent water loss except by transpiration and arranged in the greenhouse according to

93 the experimental design. Thereafter, all the pots were weighed and this weight was defined as the initial pot weight.

94 The inter-daily weight of the pots was measured for ten days to calculate the initial dry-down parameters for treatment

95 application (Fig. 1b). The WD treatment started at 45 dap which coincides with the beginning of tuber initiation.
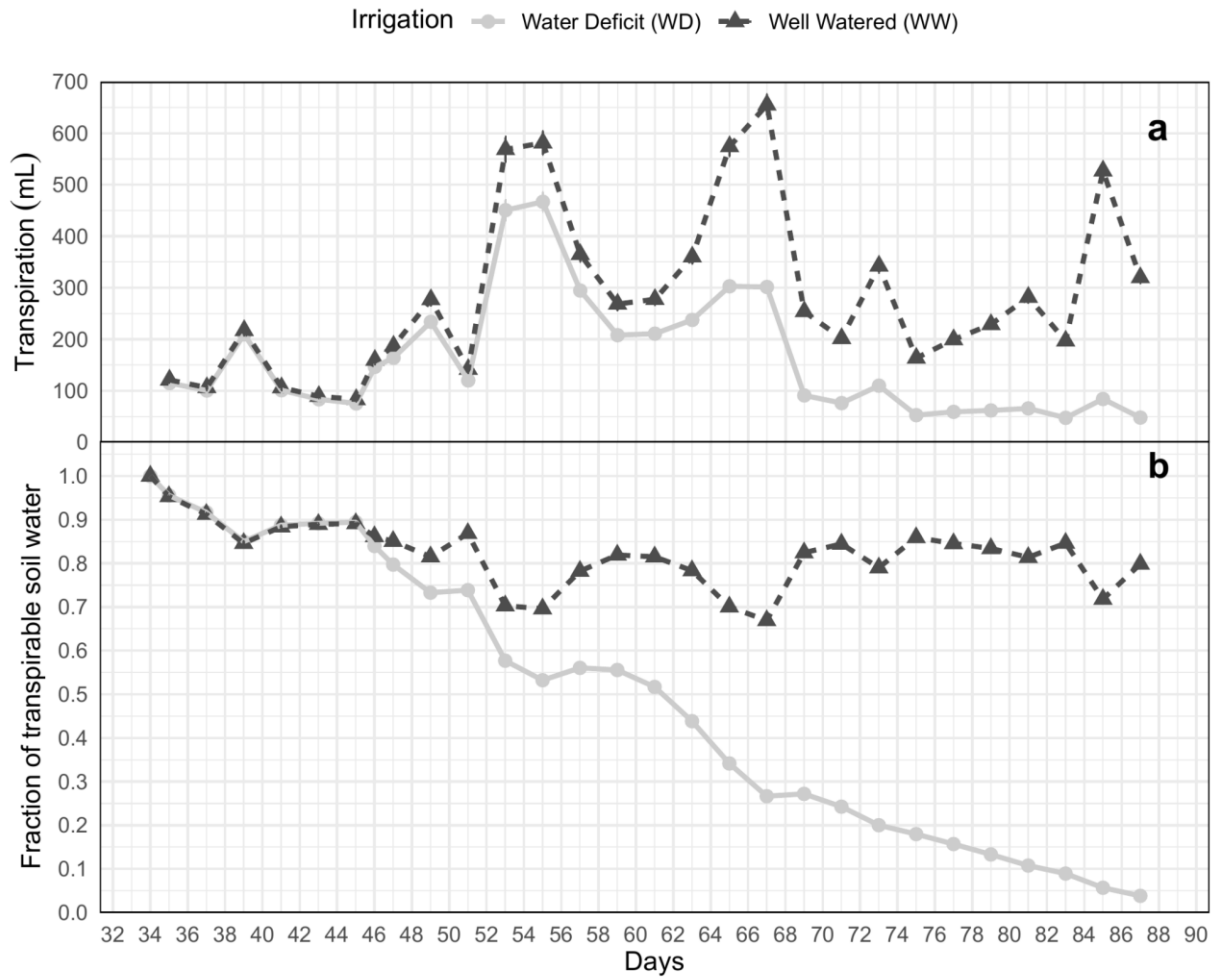
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Fig. 1: Water use efficiency in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. (a) Daily transpiration. (b) Fraction of transpirable soil water (FTSW).

Transpiration rate

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

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

The normalization of the transpiration (TRS) was achieved by dividing the transpiration of each plant in the WD regime ($WD_{transpiration}$; mL) by the mean transpiration of the WW plants ($WW_{transpiration.average}$; mL). For comparing the transpiration between plants, a second normalization was done so that the normalized transpiration rate (NTR) of each

107   plant was defined as 1.0 when the soil water content in each pot was at field capacity (Sinclair and Ludlow 1986). The

108   available soil water or the fraction of transpirable soil water (FTSW), for each pot, was calculated by dividing the pot

109   weight (g) minus the final pot weight by the transpirable soil water of that pot, using the following formula:

110
$$FTSW = \left(\frac{pot.weight_{inter-daily} - pot.weight_{final}}{pot.weight_{initial} - pot.weight_{final}}\right)$$

111   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).

112   The FTSW in WW plants was maintained above 70%, while for the WD treatment, the gradual restriction in water

113   supply decreased the water availability. The effect of the FTSW under WD was visible after 8 days of water restriction

114   was applied (Fig. 1b). The plants were harvested when the plants in WD had less than 10% of FTSW (Fig. 1b).

115   *Agro-physiological traits*

116   **Water Use Efficiency (WUE;** g/L**):** We have calculated the biomass water use efficiency (WUE$_B$) and tuber water

117   use efficiency (WUE$_T$). The WUE$_B$ was calculated as the total biomass in dry weight (g) produced divided by the

118   cumulative water transpired (Liu et al. 2006); for WUE$_T$ we used the dry weight (g) from tuber production divided by

119   the total water transpired (TRS; L) during the irrigation treatment.

120   **Relative Water content (RWC; %):** Relative water content was determined by weighing the fresh weight (leaflet$_{FW}$;

121   g) of the 3rd leaflet from the youngest fully expanded leaf in the third leaf from the apical part for each plant and then

122   placing it in a 4x3 inch Ziploc bag containing distilled water for 24 hours. Excess water was removed by blotting each

123   leaf in a paper towel before taking turgid weight (leaflet$_{TW}$; g). Leaves were reweighed after drying (leaflet$_{DW}$; g) in

124   an oven overnight at 90ºC. All the components were weighted on a precision scale (0.001 g). RWC was calculated

125   following the formula described by Vasquez-Robinet et al. (2008):

126
$$RWC(\%) = \left(\frac{leaflet_{FW} - leaflet_{DW}}{leatlet_{TW} - leaflet_{DW}}\right).100$$

127   **Leaf Osmotic Potential (LOP; MPa):** Leaf Osmotic Potential was determined using a dew point microvoltmeter

128   (HR-33T Wescor Inc., Logan, UT, USA) with leaf discs of 5 mm diameter, taken from the third fully extended leaf.

129   The leaf discs were put in 1 mL cryogenic tubes and frozen in liquid nitrogen. The frozen leaves were incubated at

130   22°C for 30 min in a sealed C-52 chamber (Wescor Inc., Logan, UT, USA).

131   **Relative chlorophyll content (SPAD):** Relative chlorophyll content of leaves was evaluated by taking SPAD (Soil

132   Plant Analysis Development) measurements using a SPAD-502 chlorophyll meter (Konica Minolta Sensing, Inc.,

133   Osaka, Japan) from the youngest fully expanded leaf in the third leaf from the apical part in three different points on

134   the leaflet. Individual readings of leaflets were averaged to represent the individual measurement of a leaf. The SPAD

135   was taken throughout plant development at 29, 59, 76, and 83 dap. At 29 dap all plants were before the stress was

136   imposed.

137   **Morphological traits:** The harvest was performed at 90 dap when the plants in WD had less than 10% of FTSW. The

138   plant height (HGT, cm) was recorded from the base of the soil to the top of each plant with a measuring tape. After

139 that, each plant was cut to the soil level and washed to remove all substrate. The tuber number (NTUB) and the root
140 length (RTL, cm) were recorded. In the case of TDW, all the tubers were chopped before being set in the oven. Each
141 plant was separated into four components: leaves, stems, roots, and tubers. The leaf area (LFA; cm^2) was measured
142 with the fresh leaves. The leaves were arranged on a wooden board and they were photographed. Each picture was
143 analyzed using ImageJ software (Zárate-Salazar et al. 2018).

144 **Post-harvest evaluations:** The dry weight of leaves (LDW; g), stems (SDW; g), roots (RDW; g), and tubers (TDW;
145 g) was determined with a precision scale (0.01 g) after drying all the components individually in kraft bag paper at
146 80°C for three days in a forced-air oven. The total dry biomass (TDB; g) was calculated with the sum of all
147 components.

148 **Indices:** The harvest index (HI) was calculated as the ratio of TDW related to the TDB. The specific leaf area (SLA;
149 cm^2/g) was calculated by dividing LFA by LDW. The relative chlorophyll content (RCC) was determined by
150 calculating the ration between SPAD at 83 dap and LFA.

151 *Statistical analysis*

152 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
153 al. (2018). Where μ is the intercept and G_i, B_j and ϵ_{ij} are normally distributed random effects for genotype, block, and
154 residuals, respectively. Variance components were estimated by restricted maximum likelihood implemented in the
155 H2cal() function implemented in the *inti* package (Lozano-Isla 2023). Plot-based heritability was estimated by: $H^2 =$
156 $\frac{\theta_G^2}{\theta_G^2 + \theta_\varepsilon^2}$. Where θ_G^2 and θ_ϵ^2 are the variance components for genotype and residual, respectively.

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

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

165

166

167 **Results**

168 *Agronomic and physiological traits response to water deficit*

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

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

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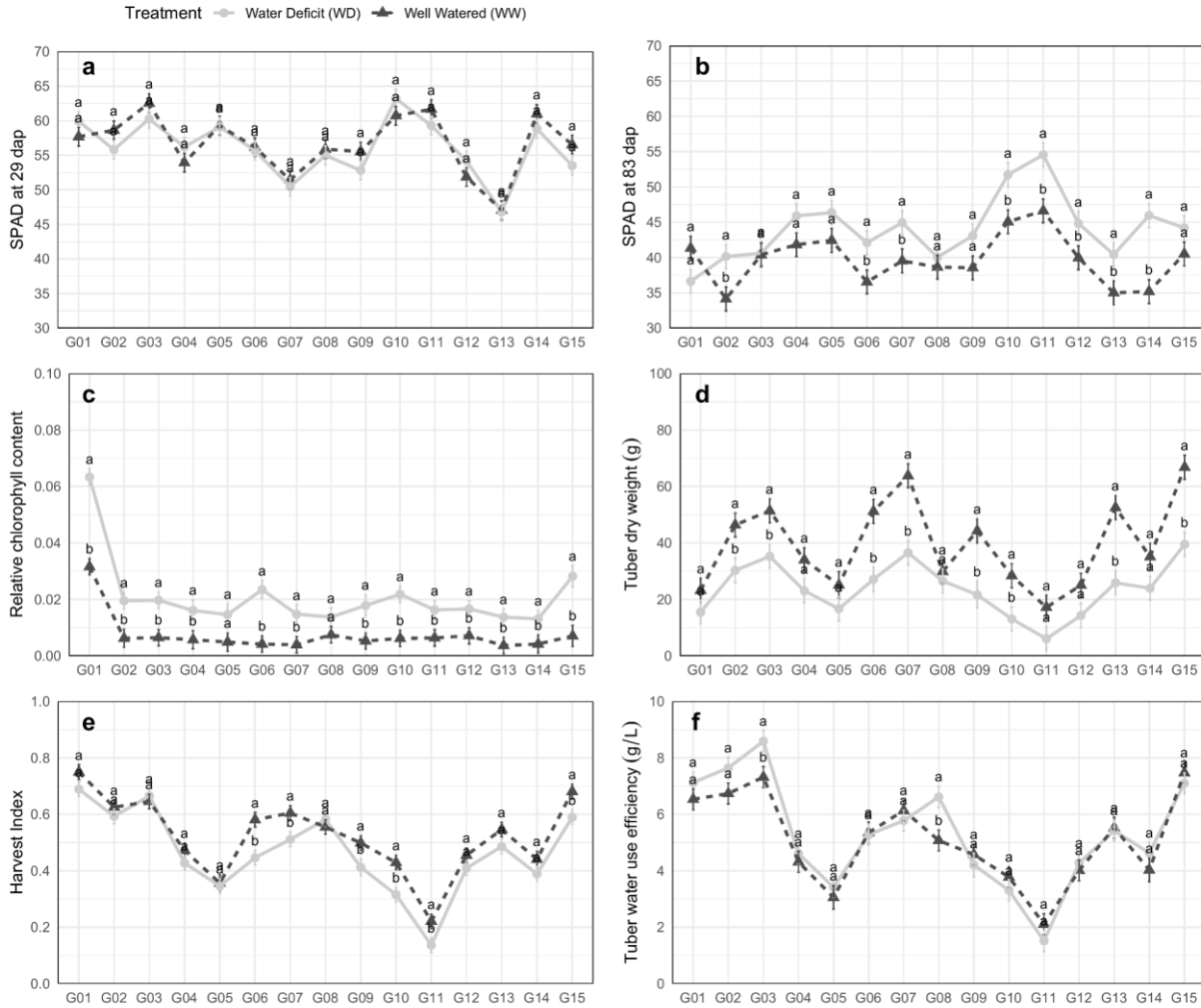183 and CIP398208.704 with 53%, 65%, and 64% respectively.

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 letters show the differences in treatment by genotype.

184   The morphological traits HGT, LDW, SDW, and LFA decreased significantly under WD (Table 2). The LFA plants
185   presented a reduction of 65% in growth under WD compared to WW plants. (Table 2). We did not find differences
186   between the irrigation treatment for NTUB, RDW, and RTL (Table 2). The RCC allowed discriminating genotypes
187   between WD and WW (Fig. 2c). The genotypes with best performance for RCC were CIP720088 (Achirana-INTA),
188   CIP398208.620, CIP398208.704, CIP398201.510, CIP392797.22 (UNICA) and CIP397077.16 (Fig. 2c, Table S1).

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

Variable	Water deficit	Well-Watered	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 (HGT; cm)	132 ± 15	150 ± 16	<0.001
Relative water content (RWC; %)	58 ± 6	69 ± 5	<0.001
Leaf osmotic potential (LOP; MPa)	-2.84 ± 0.30	-2.25 ± 0.29	<0.001
Leaf dry weight (LDW; g)	12.0 ± 3.7	17.3 ± 5.5	<0.001
Stem dry weight (SDW; g)	11.6 ± 9.1	14.5 ± 6.1	<0.001
Root dry weight (RDW; g)	3.67 ± 1.94	3.50 ± 1.96	0.6
Tuber dry weight (TDW; g)	24 ± 11	40 ± 19	<0.001
Tuber number (NTUB; N°)	12.0 ± 6.2	12.0 ± 4.9	0.8
Total transpiration (TRS; mL)	4.52 ± 1.22	7.85 ± 2.20	<0.001
Leaf area (LFA; cm^2)	2,488 ± 797	7,100 ± 2,380	<0.001
Root length (RTL; cm)	33.1 ± 6.5	32.5 ± 5.8	0.4
Total dry biomass (TDB; g)	51 ± 16	75 ± 24	<0.001
Harvest index (HI)	0.47 ± 0.16	0.53 ± 0.14	0.020
Specific leaf area (SLA; cm^2 g^{-1})	218 ± 62	415 ± 82	<0.001
Relative chlorophyll content (RCC)	0.021 ± 0.015	0.008 ± 0.007	<0.001
Biomass water use efficiency (WUE~B~; g L^{-1})	11.32 ± 2.15	9.53 ± 1.26	<0.001
Tuber water use efficiency (WUE~T~; g L^{-1})	5.31 ± 2.03	5.09 ± 1.75	0.5

192

193   The TDB for WD reduced by ca. 32% in comparison with the WW treatment (Table 2, Fig. 3). In terms of productivity,

194   WD treatment decreased TDW across genotypes by ca. 40% (Fig. 2d). The genotype CIP398190.89 had greater TDW

195   in WD treatment compared to its yield in WW treatment with a 5% increase in biomass, while other genotypes like

196   CIP398203.5 and CIP398203.244 presented up to 56% and 48% reduced tuber production (Fig. 2d). The genotypes

197   CIP398203.244, CIP398180.612, and CIP398201.510 were among the most sensitive genotypes at 31.6%, 46.7%, and

198   48.9% respectively under WD (Fig. 2d, Table S1).

199   Significant differences were found for HI among genotypes and treatments (Fig. 2e, Table 2). Genotypes showed HI

200   at ca. 53% under WW conditions and the water deficit was reduced by 11% (Table 2). WUE_B was generally higher

201   for genotypes under WD treatment than under WW treatment (Table 2). Under WD treatment, CIP397077.16,

202   CIP398208.620, CIP392797.22, and CIP398190.89 showed the highest WUE_B with 13.06, 12.03, and 11.59 g/L,

203   respectively. The lowest WUE_B with 8.50 and 9.24 g/L were presented by CIP398180.612 and CIP398203.5,

204   respectively. For WUE_T there is no significant difference between treatments (Table 2). The genotypes with higher

205   WUET under WD treatment were CIP397077.16, CIP392797.22 (UNICA), CIP720088, and CIP398208.620 (Fig. 2f,
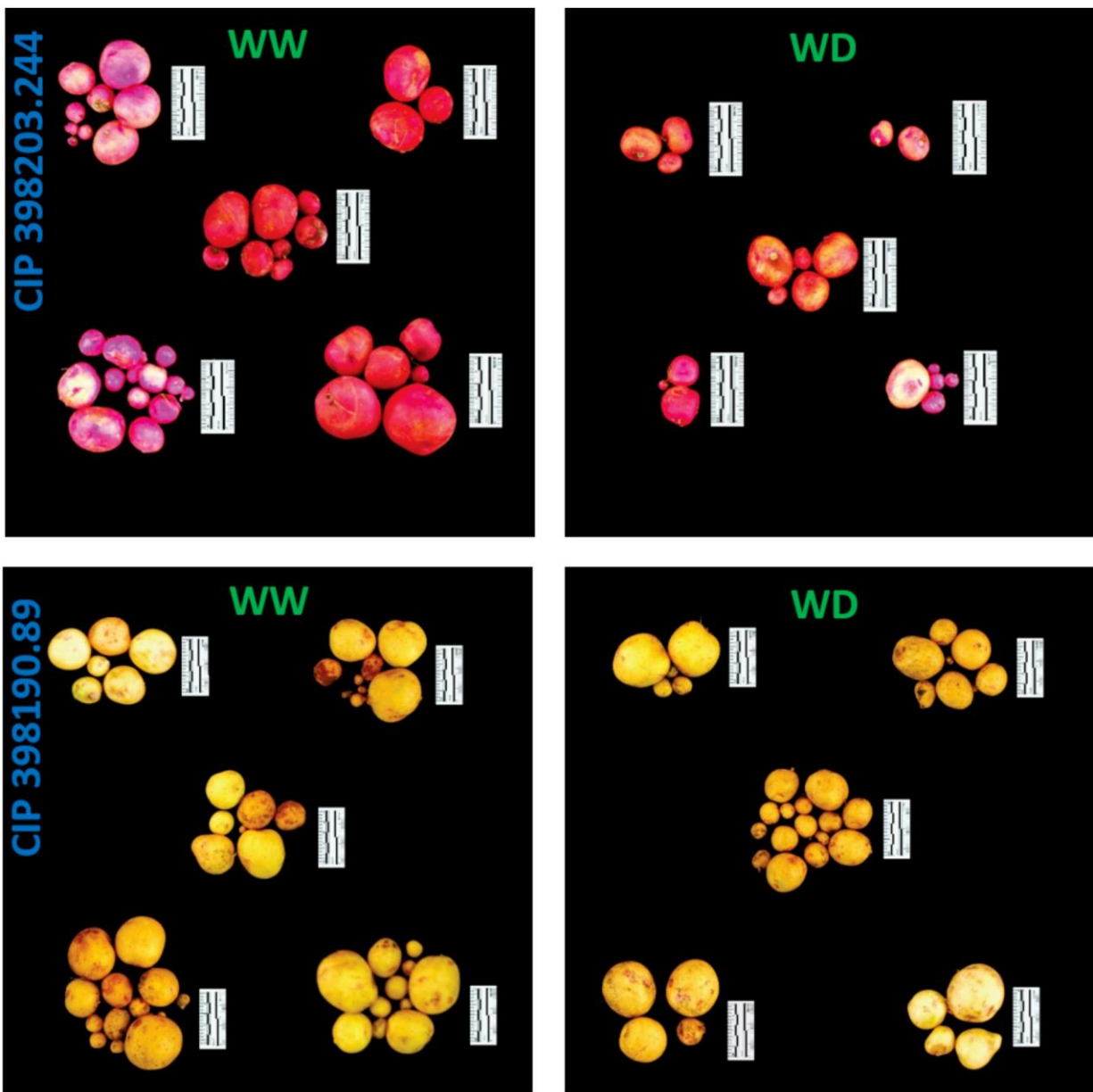
206   Table S1).

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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.

209

Genotype selection under water deficit

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

215 The TDW had a positive correlation with TDB (r = 0.84), HI (r = 0.61), and TRN (r=0.71) and a consistent negative
216 correlation in the three measurements of SPAD at 83 dap (r = -0.60) (Fig. 4). LFA showed a high correlation with
217 TRS (r = 0.93) and LDW (r = 0.85) while presenting a negative correlation with RCC (r = -0.76) (Fig. 4). The HI
218 presents a negative correlation with SPAD at 83 dap (r = -0.7), RDW (r = -0.73), and RTL (r = -0.75); while WUE_T
219 showed a strong positive correlation with HI (r = 0.92) and negative correlation with RTL (r = -0.73). The RCC
220 presented a negative correlation with TRS (r = -0.76), LFA (r = -0.76), and LDW (r = -0.78) (Fig. 4).
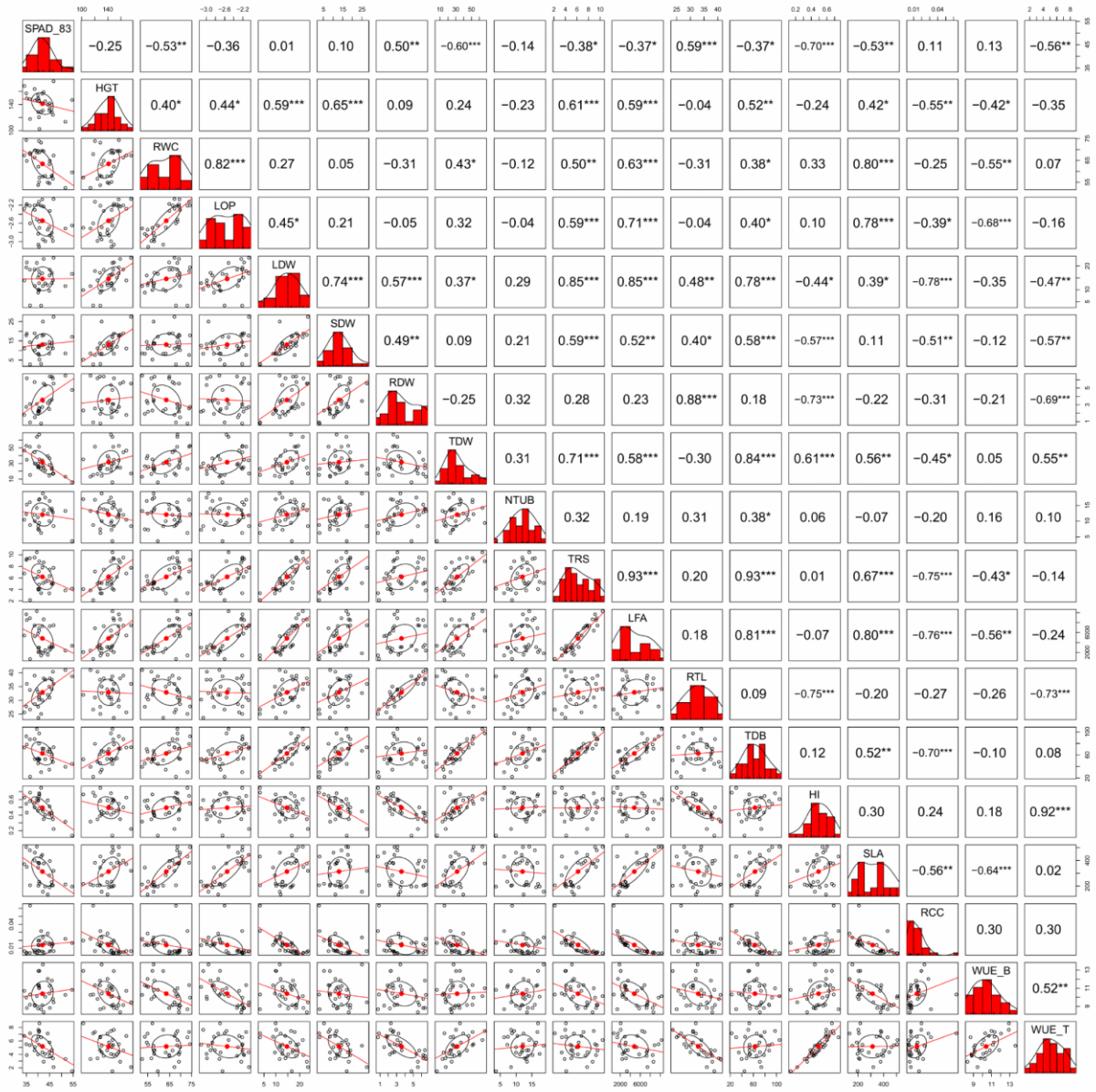
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Fig. 4: Pearson correlation among agronomic and physiological traits evaluated in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. Correlation coefficients (above diagonal), linear regressions (below diagonal), and trait 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^2), Root length (RTL; cm), Total dry biomass (TDB; g), Harvest index (HI), Specific leaf area (SLA; $cm^2\ g^{-1}$), Relative chlorophyll content (RCC), Biomass water use efficiency (WUE$_B$; g L^{-1}), Tuber water use efficiency (WUE$_T$; g L^{-1}).

223 To understand the interaction and response of the genotypes under WW and WD conditions a PCA for the traits and

224 individuals was performed. The first two components in the PCA explained 64.9% of the variance (Fig. 5, Fig S1a).

225 In the first dimension, the five traits with the highest contribution were TRS, LFA, TBD, SLA, and RCC (Fig. S1b).

226 In the second dimension, the traits with major contributions were HI, WUE$_T$, RTL, RDW, and SPAD 83 dap (Fig.

227 S1c). In the first dimension, there was a positive correlation between LDW, LFA, and TRS with a negative correlation
228 with RCC and WUE_B (Fig. 5a, Fig. S1d). The genotypes with high LFA presented more TRS and LDW but they have
229 low RCC and WUE_B (Fig. 5a, Fig. S1d). In the second dimension, RTL and RWD were correlated and presented
230 negative correlations with HI and WUE_T (Fig. 5a, Fig. S1d). The plot-based heritability presented high values for HI
231 (0.96), RDW (0.95), WUE_T (0.94), SDW (0.89), and RTL (0.83). And, low values for RWC (0.07), LFA (0.44), SLA
232 (0.03), and OP (<0.001) (Fig. 5a, Table S2)

233 The PCA for the individuals grouped the genotypes in five clusters (Fig. 5b). Cluster 1 was associated with the
234 genotype Achirana-INTA with early maturity (Table 1). Clusters 2 and 3 are associated with the genotypes under WD
235 conditions; while clusters 4 and 5 are related to the genotypes under WW conditions. In the distribution between the
236 individuals and traits, the genotypes UNICA, CIP397077.16, CIP398190.89, and CIP398208.620 are located in
237 clusters 3 and 5 presented higher performance under WD conditions with high WUET, HI, and RCC (Fig. 5, Fig. 2c,
238 e-f). The genotypes CIP398203.244, CIP398180.612, CIP398201.510, and CIP398192.592 located in clusters 2 and
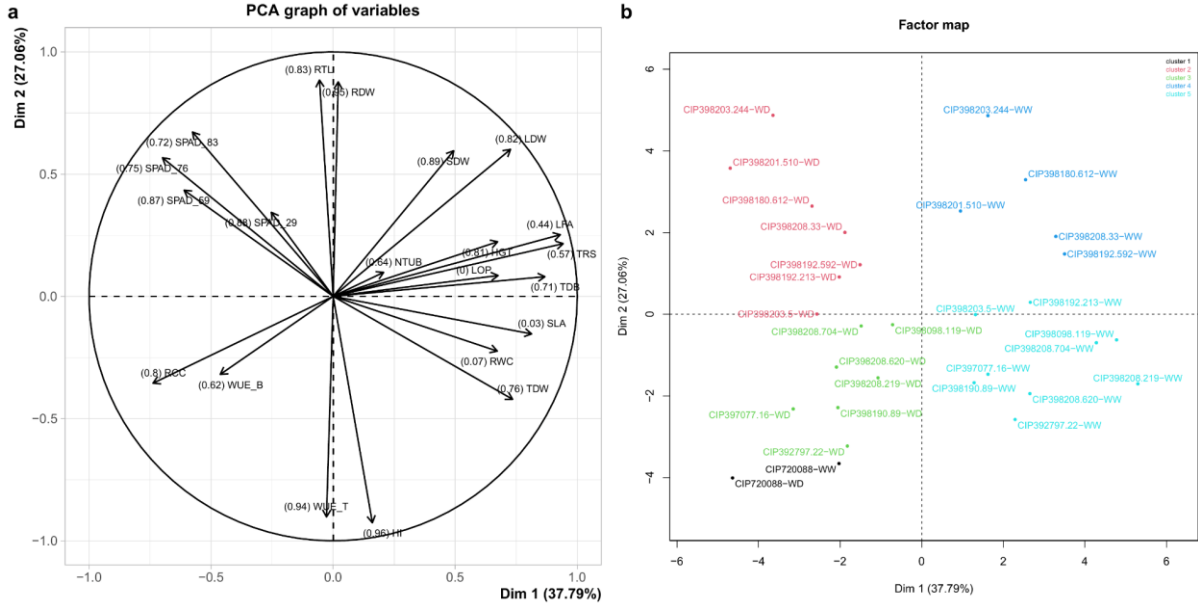239 4 presented low performance under both treatments (Fig. 5b, Fig. S2).

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Fig. 5: Principal Component Analysis (PCA) from traits measured in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. (A) PCA for the 18 traits. (B) PCA for 15 potato genotypes under WW and WD conditions. Plot-based heritability values are in brackets. 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^2), Root length (RTL; cm), Total dry biomass (TDB; g), Harvest index (HI), Specific leaf area (SLA; cm^2 g^{-1}), Relative chlorophyll content (RCC), Biomass water use efficiency (WUE$_B$; g L^{-1}), Tuber water use efficiency (WUE$_T$; g L^{-1}).

241

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, Fig. 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

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

282 One significant limitation of this study is that it was conducted in a single experiment, which may not fully capture
283 the impact of environmental variability on complex traits such as drought tolerance. Nonetheless, the study addressed
284 this limitation by conducting the experiment under controlled conditions and using the lysimeter method to calculate
285 water consumption by a plant which would be difficult to do under field conditions. Additionally, the plot-based
286 heritability was estimated for the studied traits (Fig. 5a). The limited space in the greenhouse prevented the inclusion
287 of additional pots to adjust water requirements over time for each treatment. However, the study successfully achieved
288 the difference between the water deficit treatment and stress conditions (Fig. 1), with all pots having the same source
289 of variation and normally distributed errors. To obtain more reliable insights into the variability and stability of drought
290 tolerance traits in potato genotypes, future investigations involving multiple environmental trials and a larger sample
291 size would be necessary.

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

298

Data availability

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

Conflict of interest

The authors declare no conflicts of interest.

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