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

Manuscript Number:	JCSB-D-23-00038R1	
Full Title:	Harvest index is a key trait for screening drought-tolerant potato genotypes (<i>Solanum tuberosum</i>)	
Short Title:	HI to screening drought-tolerant potato genotypes	
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Abstract:	<p>Drought stress is a significant constraint that affects the yield of almost all major crops. Its impact is expected to worsen due to global climate change and human population expansion. Potato (<i>Solanum tuberosum</i> L) is the third most important food crop worldwide, but it is highly susceptible to drought stress. In order to understand the effects of drought stress on yield and identify traits for selecting 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) was found to have a high plot-based heritability (0.98), indicating that it can be used for the indirect selection of drought-tolerant genotypes. In contrast, SPAD was sensitive to detecting early drought stress in potatoes. Drought-tolerant genotypes, including CIP392797.22 (UNICA), CIP397077.16, CIP398190.89, CIP398208.219, and CIP398208.620, were able to allocate limited water towards tuber production rather than biomass. These genotypes showed high tuber production under WW conditions and increased photosynthetic activity and water use efficiency under WD conditions.</p>	
Response to Reviewers:	Response to Reviewer 1.	
	<p>Reviewer #1: In this manuscript, the authors evaluated various agronomic traits of 15 potato genotypes under well-watered and water-deficit conditions and identified traits that can be used for selecting drought tolerant genotypes. Overall, the manuscript is well-written. Please consider the comments below to improve the manuscript.</p>	
	<p>R.: The authors are grateful for the recommendations and suggestions provided for our manuscript. In response to your inquiries, we have implemented improvements to</p>	

enhance the manuscript.

1) I think the major limitation of this study is that phenotyping had been carried out in only one experiment almost 10 years ago in 2013. A single environment trial is obviously insufficient to evaluate a complex trait such as drought tolerance, which is greatly affected by environmental variation. This should be mentioned in the discussion section along with the limitation pointed out by the authors in line 267-270.

R.: The suggested limitations were included in the manuscript along with how they were addressed to try to overcome them, as well as recommendations for future research (Line 282-291).

2) There are so many abbreviations used for different traits. Please add these in Table 2 for the convenience of the readers.

R.: The abbreviations were added to the Table 2 for better readability.

3) There are many typos and grammar errors across the manuscript. For example, in the title page,

- line 1: "Harvest index is a key..." instead of "Harvest Index a key..."

- line 7: "... potato genotypes, we evaluated..." instead of "... potato genotypes. We evaluated..."

- line 8: "The harvest index", not "The Harvest index"

Please check carefully throughout the manuscript and correct the errors and typos.

R.: The title was correct for: "Harvest index is a key trait for screening drought-tolerant potato genotypes (*Solanum tuberosum*)". The errors and typos were corrected across the document.

Response to Reviewer 2.

Reviewer #2: This is the review for the manuscript titled "Harvest index a key trait to select tolerant potato genotypes under drought stress condition".

R.: The authors express their sincere gratitude for the comprehensive manuscript analysis conducted. We confirm that all the points highlighted in the review have been addressed and corrected, denoted in red. Additionally, a thorough proofreading has been conducted. Thank you for your valuable contributions to enhancing this manuscript.

1. Title is not clear "select tolerant"????

R.: We have enhanced the title by incorporating the following changes: "Harvest index is a key trait for screening drought-tolerant potato genotypes (*Solanum tuberosum*)".

2. In the introduction, previous research background associated with the objective of the study (1) is not sufficient. Please provide physiological background more.

R.: We include a paragraph in the introduction about the responses in morphological, physiological, biochemical, and molecular pathways under drought stress in potato (Line 33-49).

3. Objective of the study (2) is not clear, reword please.

R.: The objectives have been rewritten to enhance clarity and comprehension (Line 57-61).

4. "International Potato Center (CIP)" Line 54, and 57. What is the point of having abbreviation?

R.: The abbreviation is only included in the first mention. We think that the abbreviation is important as made a reference to the institution on the genotype's name (Line 66).

5.	Line 53, did it mean CIP bred those varieties?
	R.: Yes, the genotypes used are part of the advanced breeding population from CIP.
6.	It has been ten years since the research undertaken. I do not understand why it took ten years old one year study.
	R.: The present study was conducted as part of a thesis, and due to its significance, the institution has recently recommended its publication. Although the results were obtained a few years ago, the experiment was conducted in a greenhouse under controlled conditions using the lysimeter method. It should be noted that this research served as a preliminary assessment for conducting subsequent experiments under field conditions. We includes this information in the limitations section (Lines 282-291).
7.	Line 63 need converted amount of fertilizer in area base /ha. In addition, this is not right way to show NPK input ratio. It should be either NPK or N, P2O5, K2O. So what was NPK input???? Application method also should be indicated.
	R.: The information was added to the M&M (Line 75-80).
8.	Line 72, RCBD?? I am not sure why they chose RCBD nor CRD in greenhouse pot experiment
	R.: Since we have six tables in the greenhouse, we opted to use each table as a block in the randomized complete block design (RCBD) arrangement for the experiment.
9.	Fig. 1 a,b graph type is somewhat different.
	R.: The Fig. 1 was updated with the same space between x-labels and corrected the legend size.
10.	Line 134, I do not understand how RCC was calculated.
	R.: The sentence has been rewritten for better understanding
11.	Line 160, reword "the ones"
	R.: The sentence has been rewritten for better understanding.
12.	In the text use abbreviation for figure (fig.)
	R.: The prefix for Figure was changed across the manuscript to Fig.
13.	Line 169, There was~~~ please reword for better understanding.
	R.: The sentence has been rewritten for better understanding
14.	Figure 2, a, b why only those show as line not bar?
	R.: To emphasize the difference between SPAD measurements before and after drought stress, we increased the y-axis to focus on the range of 30 to 70 units. This allowed for easier comparison of the results. To maintain consistency across all subfigures, we decided to use line plots instead of bar plots.
15.	Fundamental error of figure 2. It did not show statistical differences with current manner. Where can I read difference?? While there is explanations in Line 172 and after??? Are you comparing genotype? Before and after? Or both??
	R.: The figures now include the significance of the comparison between each treatment by genotype. However, due to the high number of genotypes per treatment, visualizing this information becomes challenging. To address this, we have included a supplementary table (Table S1) specifically for the comparison of genotypes within each treatment.
16.	Line 186, just curious WUE is L/g not g/L in common way?? So I can know how

much water is used to produce 1 gram?

R.: Water use efficiency is typically expressed in different units depending on the context and the specific measurement being used. Grams per liter (g/L): This unit is often used for measuring water use efficiency under laboratory experiments or controlled conditions. Based in the observation, we include the following reference in the M&M for WUE description:

Liu, F., Shahnazari, A., Andersen, M. N., Jacobsen, S.-E., & Jensen, C. R. (2006). Physiological responses of potato (*Solanum tuberosum* L.) to partial root-zone drying: ABA signalling, leaf gas exchange, and water use efficiency. *Journal of Experimental Botany*, 57(14), 3727-3735. <https://doi.org/10.1093/jxb/erl131>

17. Line 234 and after, Yes HI is good indicator for water deficit associated yield loss. That is because to calculate the HI, yield is needed anyway. Index with yield represent yield does not make sense to me. I may misunderstand concept. But so should readers. Please explain how it make sense.

R.: While assessing water use efficiency under field conditions is challenging and not easily estimated, the harvest index (HI) is commonly linked to yield. Nevertheless, the findings of this study illustrate that HI can be a valuable tool for screening genotypes with drought tolerance. Additionally, HI is easily assessable in a large number of genotypes and shows a high level of heritability (Fig 5a, Table S2).



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

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

18 Author contributions

19 Conception and design of the study by KA, RG. Material preparation, data collection, and analysis were performed
20 by FLI, ERFV, RG, RB. The first draft of the manuscript was written by FLI and ERFV and the authors commented
21 on previous versions of the manuscript. All authors read and approved the final manuscript.

22 Acknowledgments

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24 and water management technologies to enhance water use efficiency, resilience, cost-effectiveness, and productivity
25 of smallholder farms in stress-prone Central Asian environments”. We also thank Jorge Vega and David Saravia for
26 their support during the installation and evaluation of the experiments.

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

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.

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

18 Introduction

19 Drought stress affects agricultural productivity worldwide and is expected to rise in the coming years (Hasegawa et
20 al. 2021). Breeding crops with increased yield potential and improved tolerance to stressful environments is critical
21 for global food security (Raza et al. 2022). The present crop production is insufficient to feed the growing human
22 population by 2050 (Hickey et al. 2019). Water has become a scarce and precious resource and its efficient utilization
23 in food production is a key challenge worldwide. Specifically, yield losses due to drought are a global problem limiting
24 agricultural production (Hasegawa et al. 2021). Developing plants with a tolerance to drought relies on various aspects
25 including the drought impact, duration, intensity, crop development stage, and plant genetic potential (Varshney et
26 al. 2021; Seleiman et al. 2021).

27 Potatoes are the third most consumed food crop worldwide (Devaux et al. 2020), and suffer drastic losses in tuber
28 yield and/or quality due to drought stress (Hill et al. 2021). In general, the potato has a high harvest index in
29 comparison with cereals and a relatively low demand for water i.e., 400 to 600 L for 1 kg of tuber dry matter
30 (Monneveux et al. 2013; Sprenger et al. 2016). However, periodic water shortages are critical, particularly at the tuber
31 initiation stage. Therefore, potato cultivars with effective water-saving mechanisms leading to higher tolerance are
32 highly desirable (Monneveux et al. 2014).

33 Potatoes show a variety of responses in morphological, physiological, biochemical, and molecular pathways under
34 drought stress (Aliche et al. 2022). Water deficit induced by drought is a significant abiotic stress factor that has
35 detrimental effects on potato growth, development, and tuber yield (Soltys-Kalina et al. 2016; Sprenger et al. 2016).
36 To address water scarcity, potato plants have developed various physiological, biochemical, and molecular
37 mechanisms. These mechanisms enable them to optimize water use efficiency, maintain cellular homeostasis, and
38 minimize oxidative damage. Potato plants display several physiological responses aimed at mitigating the negative
39 impacts of drought. These responses include stomatal closure, which reduces transpirational water loss, and changes
40 in leaf morphology, such as a decrease in leaf area and an increase in leaf thickness, to limit water vapor diffusion
41 (Deblonde and Ledent 2001; Joshi et al. 2016). Additionally, potatoes undergo adjustments in their root system
42 architecture, promoting deeper and more extensive root growth to access water from deeper soil layers (Khan et
43 al. 2016). In terms of molecular and biochemical mechanisms, they involve the accumulation of osmoprotectants, such
44 as proline, sugars, and compatible solutes (Obidiegwu et al. 2015). These substances help maintain cellular osmotic
45 potential and protect macromolecules from damage caused by dehydration. Antioxidant defense systems are also
46 upregulated, including the synthesis of enzymes like superoxide dismutase, catalase, and peroxidase, which scavenge
47 reactive oxygen species and prevent oxidative stress (Lal et al. 2022). Furthermore, drought-tolerant potato varieties
48 exhibit enhanced expression of stress-responsive genes involved in signal transduction, transcriptional regulation, and
49 stress tolerance pathways (Khan et al. 2015; Chen et al. 2020).

50 Water use efficiency (WUE) is an important trait for adaptation to drought stress. And it is defined as the efficiency
51 of the amount of water applied and used for transpiration that goes toward dry matter production. Enhanced WUE can
52 reduce crop water requirements and significantly increase crop yield (Yu et al. 2020). When a higher WUE under
53 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 selection of genotypes with high water use efficiency (WUE) and stable yield under drought stress are crucial steps toward achieving greater genetic gain in potatoes. The main objectives of this work were: (1) To investigate the drought tolerance mechanisms and physiological responses of potatoes under water deficit conditions, and (2) To identify traits associated with drought tolerance and high WUE that would enable the selection of genotypes capable of maintaining productivity even under limited water availability.

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 P₂O₅, and 75 kg/ha of K₂O. Nitrogen was applied in the form of ammonium nitrate (34.5% N), phosphorus was provided as triple superphosphate (45% P₂O₅), and potassium was delivered using potassium sulphate (50% K₂O).

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

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.

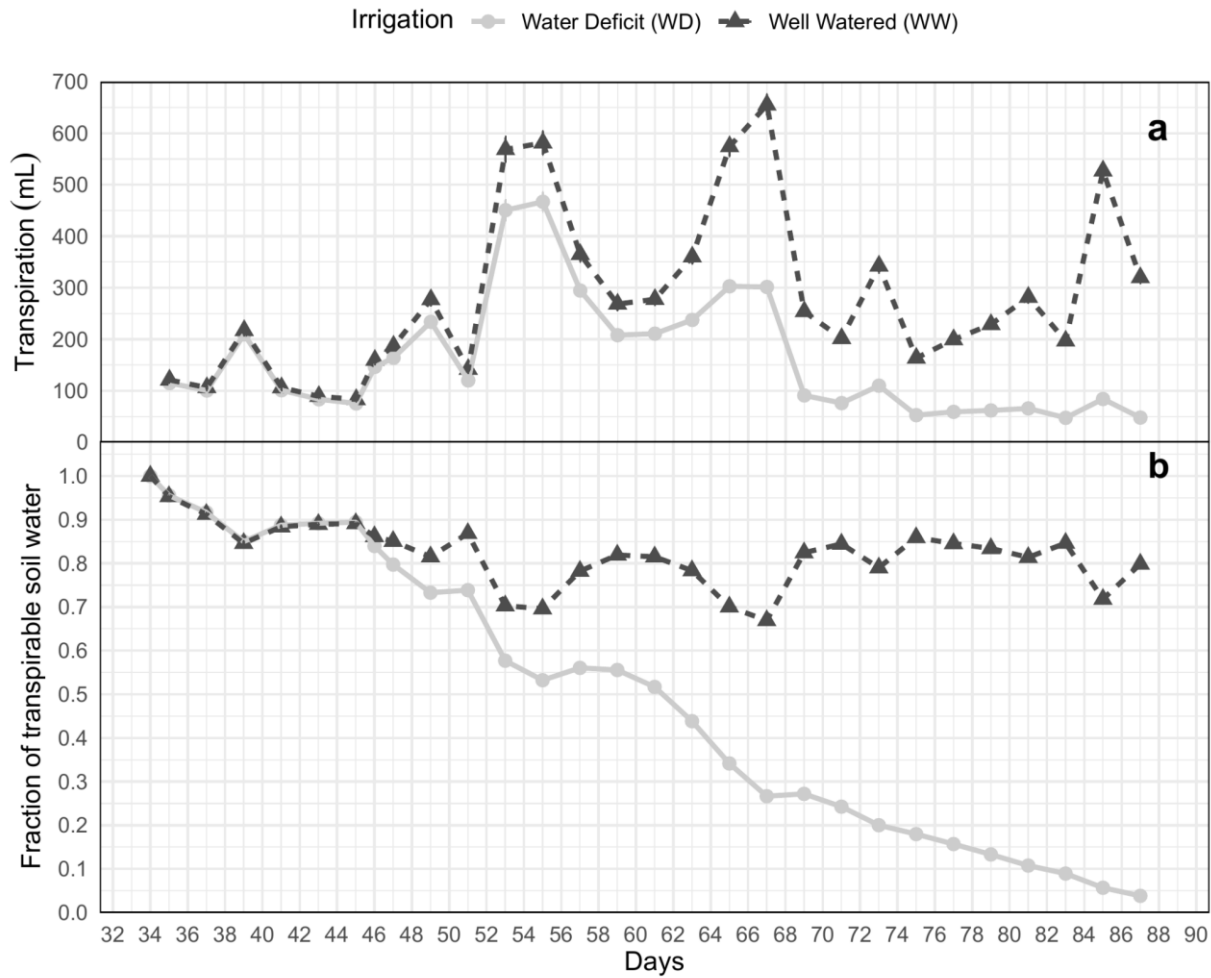


Fig. 1: Water use efficiency in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. (a) Daily transpiration. (b) Fraction of transpirable soil water (FTSW).

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

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 (Liu et al. 2006); 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 ($leaflet_{FW}$; 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 ($leaflet_{TW}$; g). Leaves were reweighed after drying ($leaflet_{DW}$; 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; cm²) 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; cm²/g) was calculated by dividing LFA by LDW. The relative chlorophyll content (RCC) was determined by calculating the ration between 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 μ is the intercept and G_i , B_j and ϵ_{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 θ_G^2 and θ_ϵ^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 (Fig. 2a); while SPAD at 83 dap showed differences between treatment and genotypes (Fig. 2b). The SPAD values were lower at 83 than at 29 dap (Fig. 2a-b, Table 2). The SPAD under WD were higher for all genotypes compared to the values under WW conditions (Table 2, Fig. 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 (Fig. 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.

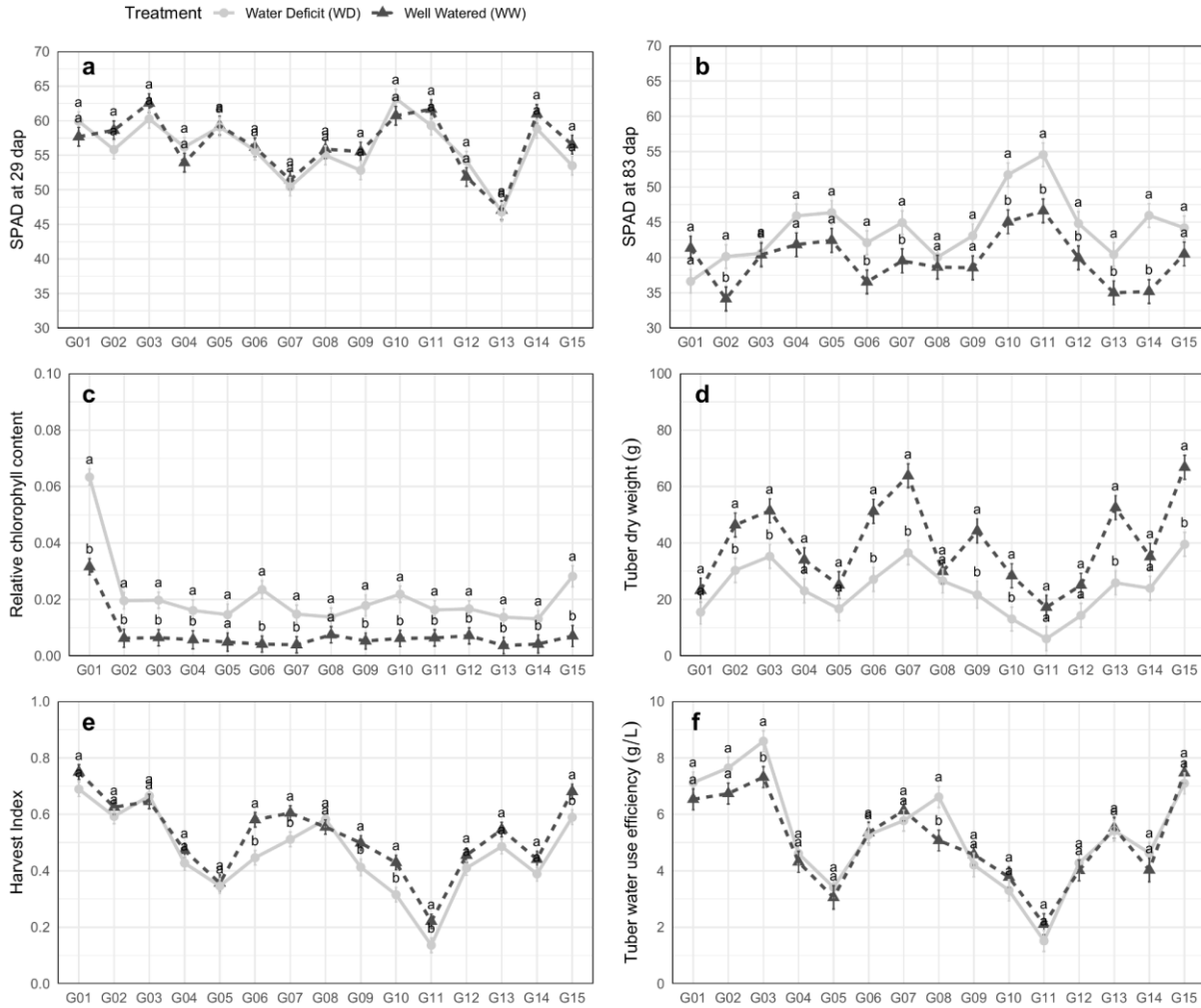


Fig. 2: Traits measured in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. (a-b) Chlorophyll concentration. (c) Tuber dry weight. (d) Relative chlorophyll content. (e) Harvest Index. (f) Tuber Water Use Efficiency. Error bars indicate standard error (n = 5). Days after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

The morphological traits HGT, LDW, SDW, and LFA decreased significantly under WD (Table 2). The LFA plants presented a reduction of 65% in growth under WD 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 (Fig. 2c). The genotypes with best performance for RCC were CIP720088 (Achirana-INTA), CIP398208.620, CIP398208.704, CIP398201.510, CIP392797.22 (UNICA) and CIP397077.16 (Fig. 2c, Table S1).

Table 2: Response comparison for 18 traits in 15 potato genotypes under Well-Watered (WW) and Water Deficit (WD) conditions. The values for different traits are represented by the mean \pm standard deviation with the significance 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 (HGT; cm)	132 \pm 15	150 \pm 16	<0.001
Relative water content (RWC; %)	58 \pm 6	69 \pm 5	<0.001
Leaf osmotic potential (LOP; MPa)	-2.84 \pm 0.30	-2.25 \pm 0.29	<0.001
Leaf dry weight (LDW; g)	12.0 \pm 3.7	17.3 \pm 5.5	<0.001
Stem dry weight (SDW; g)	11.6 \pm 9.1	14.5 \pm 6.1	<0.001
Root dry weight (RDW; g)	3.67 \pm 1.94	3.50 \pm 1.96	0.6
Tuber dry weight (TDW; g)	24 \pm 11	40 \pm 19	<0.001
Tuber number (NTUB; N°)	12.0 \pm 6.2	12.0 \pm 4.9	0.8
Total transpiration (TRS; mL)	4.52 \pm 1.22	7.85 \pm 2.20	<0.001
Leaf area (LFA; cm ²)	2,488 \pm 797	7,100 \pm 2,380	<0.001
Root length (RTL; cm)	33.1 \pm 6.5	32.5 \pm 5.8	0.4
Total dry biomass (TDB; 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 (SLA; cm ² g ⁻¹)	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 (WUE~B~, g L ⁻¹)	11.32 \pm 2.15	9.53 \pm 1.26	<0.001
Tuber water use efficiency (WUE~T~, g L ⁻¹)	5.31 \pm 2.03	5.09 \pm 1.75	0.5

The TDB for WD reduced by 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% (Fig. 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 (Fig. 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 (Fig. 2d, Table S1).

Significant differences were found for HI among genotypes and treatments (Fig. 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 (Fig. 2f, Table S1).

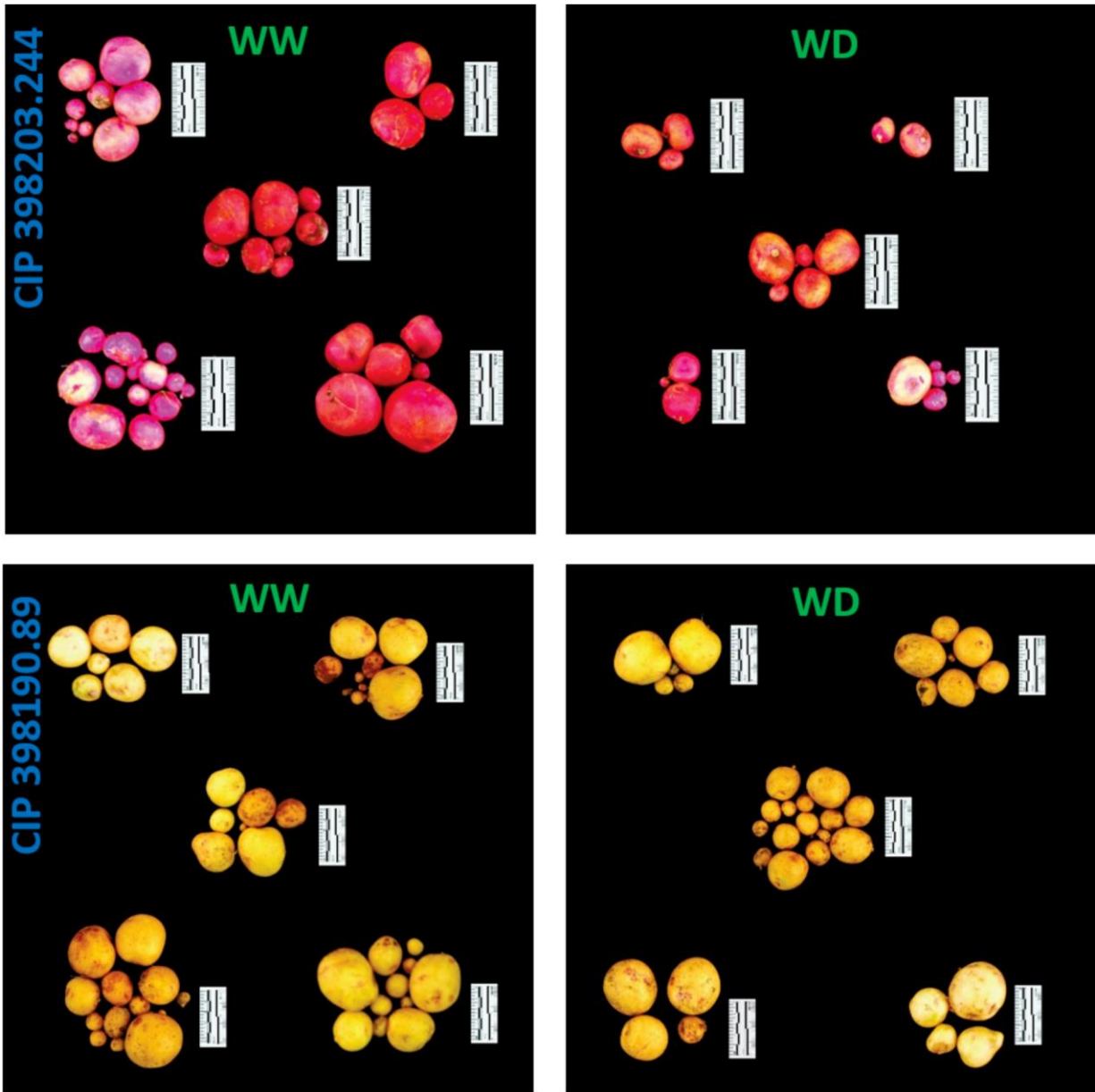


Fig. 3: Tuber yield performance in two contrasting potato genotypes under well-watered (WW) and water deficit (WD) conditions. CIP 398203.244, susceptible genotype shows good yield performance under well-watered (WW) but reduced performance under water deficit (WD) conditions. CIP 398190.89, tolerance 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).

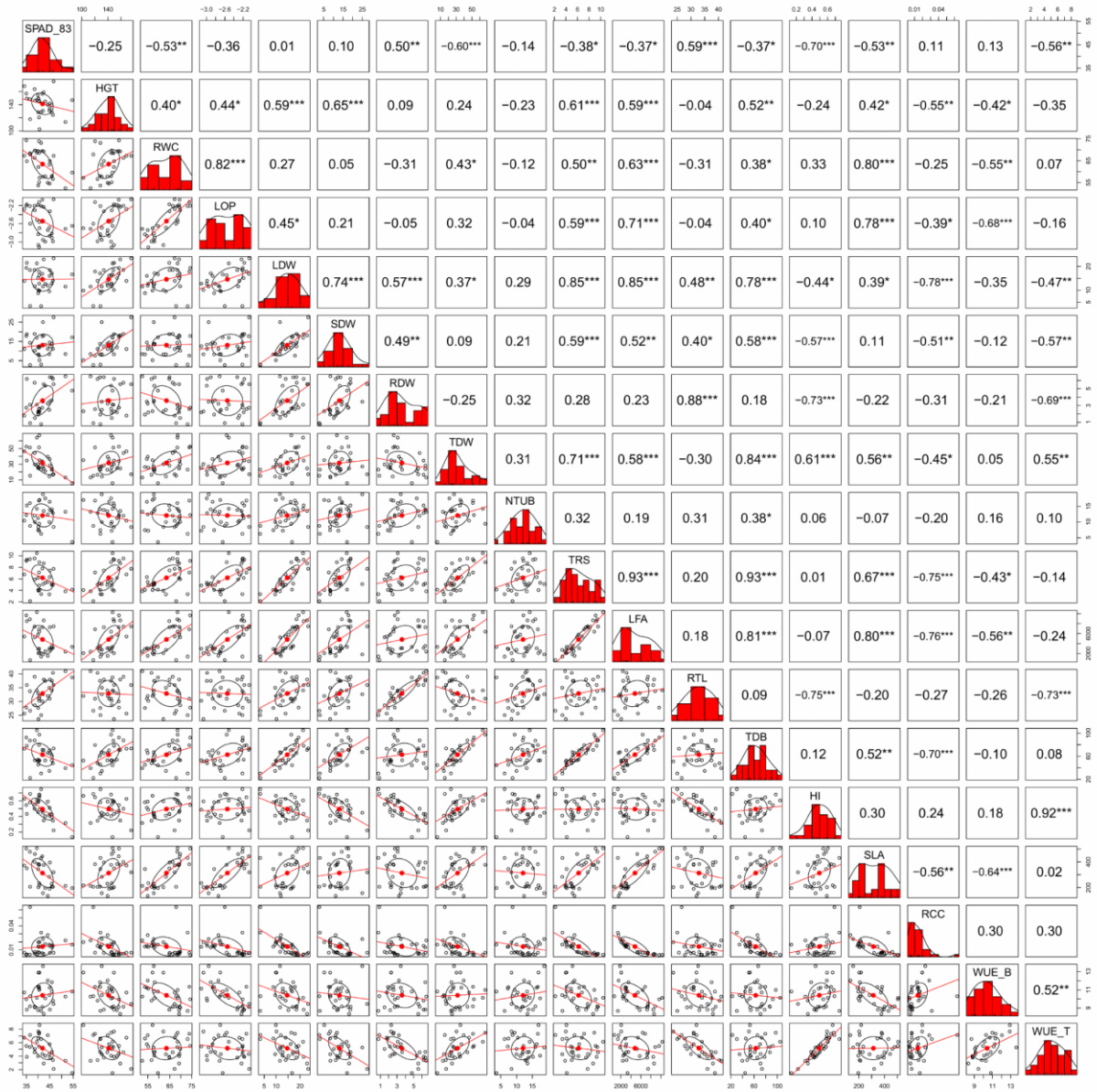


Fig. 4: Pearson correlation among agronomic and physiological traits evaluated in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. Correlation coefficients (above diagonal), linear regressions (below diagonal), and trait distribution (diagonal in red color). p-value: 0.05*, 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; cm²), Root length (RTL; cm), Total dry biomass (TDB; g), Harvest index (HI), Specific leaf area (SLA; cm² g⁻¹), Relative chlorophyll content (RCC), Biomass water use efficiency (WUE_B; g L⁻¹), Tuber water use efficiency (WUE_T; g L⁻¹).

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_T, 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_B (Fig. 5a, Fig. S1d). The genotypes with high LFA presented more TRS and LDW but they have low RCC and WUE_B (Fig. 5a, Fig. S1d). In the second dimension, RTL and RWD were correlated and presented negative correlations with HI and WUE_T (Fig. 5a, Fig. S1d). The plot-based heritability presented high values for HI (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 (0.03), and OP (<0.001) (Fig. 5a, Table S2)

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, Fig. 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).

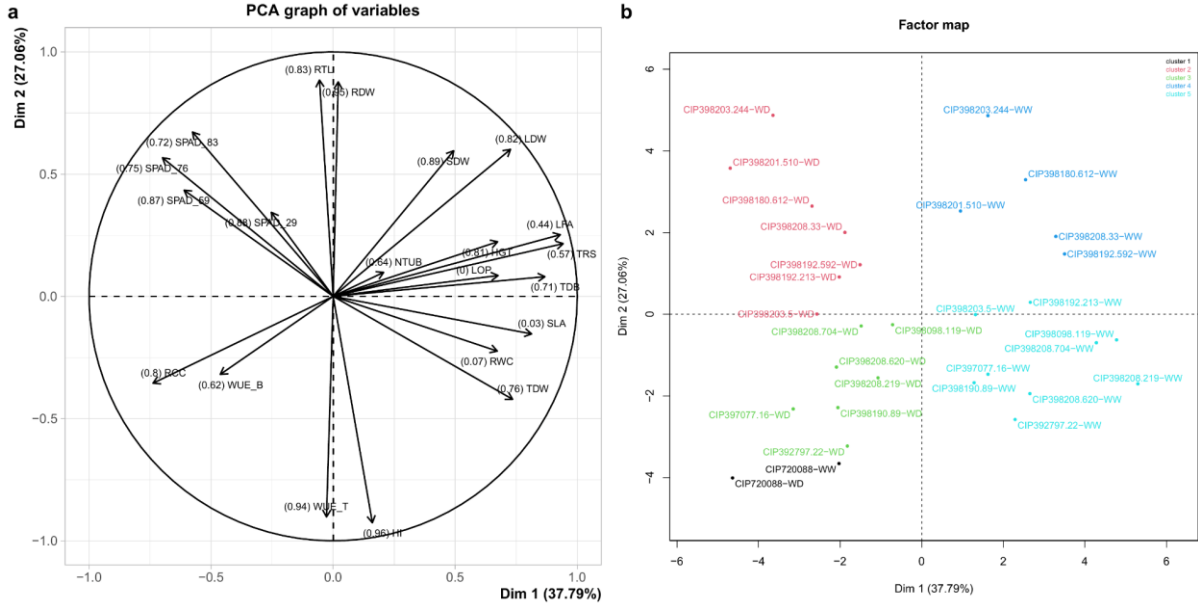


Fig. 5: Principal Component Analysis (PCA) from traits measured in 15 potato genotypes under well-watered (WW) and water deficit (WD) conditions. (A) PCA for the 18 traits. (B) PCA for 15 potato genotypes under WW and WD conditions. Plot-based heritability values are in brackets. Where: Chlorophyll Concentration (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; cm²), Root length (RTL; cm), Total dry biomass (TDB; g), Harvest index (HI), Specific leaf area (SLA; cm² g⁻¹), Relative chlorophyll content (RCC), Biomass water use efficiency (WUE_B; g L⁻¹), Tuber water use efficiency (WUE_r; g L⁻¹).

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

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.

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

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.

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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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Dr. Cheol Seong Jang
Editor-in-Chief
Journal of Crop Science and Biotechnology

La Molina, 19th May 2023


Dear Editor,

We would like to resubmit the manuscript entitled "Harvest index is a key trait for screening drought-tolerant potato genotypes (*Solanum tuberosum*)" by Flavio Lozano-Isla et al. to be considered for publication in the Journal of Crop Science and Biotechnology.

We have addressed all the suggested corrections provided by the reviewers in accordance with their recommendations. Therefore, we would appreciate a reevaluation of the manuscript. This will enable us to receive additional feedback and further enhance the quality of our work.

We think that this manuscript is highly suitable for publication in your esteemed journal and will undoubtedly be well received by the readers.

Yours sincerely,



Flavio Lozano-Isla
Corresponding author

Response to **Reviewer 1**.

Reviewer #1: In this manuscript, the authors evaluated various agronomic traits of 15 potato genotypes under well-watered and water-deficit conditions and identified traits that can be used for selecting drought tolerant genotypes. Overall, the manuscript is well-written. Please consider the comments below to improve the manuscript.

R.: The authors are grateful for the recommendations and suggestions provided for our manuscript. In response to your inquiries, we have implemented improvements to enhance the manuscript.

1) I think the major limitation of this study is that phenotyping had been carried out in only one experiment almost 10 years ago in 2013. A single environment trial is obviously insufficient to evaluate a complex trait such as drought tolerance, which is greatly affected by environmental variation. This should be mentioned in the discussion section along with the limitation pointed out by the authors in line 267-270.

R.: The suggested limitations were included in the manuscript along with how they were addressed to try to overcome them, as well as recommendations for future research (Line 282-291).

2) There are so many abbreviations used for different traits. Please add these in Table 2 for the convenience of the readers.

R.: The abbreviations were added to the Table 2 for better readability.

3) There are many typos and grammar errors across the manuscript. For example, in the title page,
- line 1: "Harvest index is a key..." instead of "Harvest Index a key..."
- line 7: "... potato genotypes, we evaluated..." instead of "... potato genotypes. We evaluated..."
- line 8: "The harvest index", not "The Harvest index"
Please check carefully throughout the manuscript and correct the errors and typos.

R.: The title was correct for: "Harvest index is a key trait for screening drought-tolerant potato genotypes (*Solanum tuberosum*)". The errors and typos were corrected across the document.

Response to **Reviewer 2.**

Reviewer #2: This is the review for the manuscript titled "Harvest index a key trait to select tolerant potato genotypes under drought stress condition".

R.: The authors express their sincere gratitude for the comprehensive manuscript analysis conducted. We confirm that all the points highlighted in the review have been addressed and corrected, denoted in red. Additionally, a thorough proofreading has been conducted. Thank you for your valuable contributions to enhancing this manuscript.

1. Title is not clear "select tolerant"????

R.: We have enhanced the title by incorporating the following changes: "Harvest index is a key trait for screening drought-tolerant potato genotypes (*Solanum tuberosum*)".

2. In the introduction, previous research background associated with the objective of the study (1) is not sufficient. Please provide physiological background more.

R.: We include a paragraph in the introduction about the responses in morphological, physiological, biochemical, and molecular pathways under drought stress in potato (Line 33-49).

3. Objective of the study (2) is not clear, reword please.

R.: The objectives have been rewritten to enhance clarity and comprehension (Line 57-61).

4. "International Potato Center (CIP)" Line 54, and 57. What is the point of having abbreviation?

R.: The abbreviation is only included in the first mention. We think that the abbreviation is important as made a reference to the institution on the genotype's name (Line 66).

5. Line 53, did it mean CIP bred those varieties?

R.: Yes, the genotypes used are part of the advanced breeding population from CIP.

6. It has been ten years since the research undertaken. I do not understand why it took ten years old one year study.

R.: The present study was conducted as part of a thesis, and due to its significance, the institution has recently recommended its publication. Although the results were obtained a few years ago, the experiment was conducted in a greenhouse under controlled conditions using the lysimeter method. It should be noted that this research served as a preliminary assessment for conducting subsequent experiments under field conditions. We includes this information in the limitations section (Lines 282-291).

7. Line 63 need converted amount of fertilizer in area base /ha. In addition, this is not right way to show NPK input ratio. It should be either NPK or N, P₂O₅, K₂O. So what was NPK input???? Application method also should be indicated.

R.: The information was added to the M&M (Line 75-80).

8. Line 72, RCBD?? I am not sure why they chose RCBD nor CRD in greenhouse pot experiment

R.: Since we have six tables in the greenhouse, we opted to use each table as a block in the randomized complete block design (RCBD) arrangement for the experiment.

9. Fig. 1 a,b graph type is somewhat different.

R.: The Fig. 1 was updated with the same space between x-labels and corrected the legend size.

10. Line 134, I do not understand how RCC was calculated.

R.: The sentence has been rewritten for better understanding

11. Line 160, reword "the ones"

R.: The sentence has been rewritten for better understanding.

12. In the text use abbreviation for figure (fig.)

R.: The prefix for Figure was changed across the manuscript to Fig.

13. Line 169, There was~~~ please reword for better understanding.

R.: The sentence has been rewritten for better understanding

14. Figure 2, a, b why only those show as line not bar?

R.: To emphasize the difference between SPAD measurements before and after drought stress, we increased the y-axis to focus on the range of 30 to 70 units. This allowed for easier comparison of the results. To maintain consistency across all subfigures, we decided to use line plots instead of bar plots.

15. Fundamental error of figure 2. It did not show statistical differences with current manner. Where can I read difference?? While there is explanations in Line 172 and after??? Are you comparing genotype? Before and after? Or both??

R.: The figures now include the significance of the comparison between each treatment by genotype. However, due to the high number of genotypes per treatment, visualizing this information becomes challenging. To address this, we have included a supplementary table (Table S1) specifically for the comparison of genotypes within each treatment.


16. Line 186, just curious WUE is L/g not g/L in common way?? So I can know how much water is used to produce 1 gram?

R.: Water use efficiency is typically expressed in different units depending on the context and the specific measurement being used. Grams per liter (g/L): This unit is often used for measuring water use efficiency under laboratory experiments or controlled conditions. Based in the observation, we include the following reference in the M&M for WUE description:

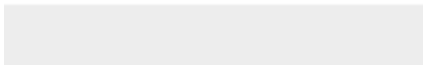

Liu, F., Shahnazari, A., Andersen, M. N., Jacobsen, S.-E., & Jensen, C. R. (2006). Physiological responses of potato (*Solanum tuberosum* L.) to partial root-zone drying: ABA signalling, leaf gas exchange, and water use efficiency. *Journal of Experimental Botany*, 57(14), 3727-3735. <https://doi.org/10.1093/jxb/erl131>


17. Line 234 and after, Yes HI is good indicator for water deficit associated yield loss. That is because to calculate the HI, yield is needed anyway. Index with yield represent yield does not make sense to me. I may misunderstand concept. But so should readers. Please explain how it make sense.

R.: While assessing water use efficiency under field conditions is challenging and not easily estimated, the harvest index (HI) is commonly linked to yield. Nevertheless, the findings of this study illustrate that HI can be a valuable tool for screening genotypes with drought tolerance. Additionally, HI is easily assessable in a large number of genotypes and shows a high level of heritability (Fig 5a, Table S2).



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