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Research article

Japanese plums behavior under water stress: impact on yield and biochemical traits

Anas Hamdani [a,b], Lahcen Hssaini [a], Said Bouda [b], Atman Adiba [a,b], Rachid Razouk [a,*]

[a] National Institute of Agricultural Research (INRA), Regional Center of Meknes, PO 578, Morocco
[b] Laboratory of Biotechnology and Valorization of Plant Genetic Resources, Faculty of Sciences and Techniques, University of Sultan Moulay Slimane, PO 523, Beni Mellal, Morocco

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ABSTRACT

This work investigates response to drought of nine local cultivars alongside two exotic varieties of Japanese plum (*Prunus salicina* L.) through their yield and fruit quality components. It was carried out at Sais plain, northern Morocco, over two consecutive years (2019–2020). Water stress was imposed by a deficit irrigation (DI) treatment of 50% ETc during the whole fruit growth period, compared to full irrigation of 100% ETc (CI). At their full ripening stage, the cultivars were assessed for their yield, fruit weight and fruit quality attributes, namely total soluble solids (TSS), pH, titratable acidity (TA), maturity index (MI), soluble sugars content (SSC), amino acids content (AAC), total phenolic compounds (TPC) and total antioxidant capacity (TAC). Results displayed significant decrease in yield and fruit weight since the first year of DI application. Owing to calculated stability indexes of the aforementioned traits along with water use efficiency, the local cultivar 'Fortu-43' was the most insensitive to drought, whereas 'Timhdit' and 'Black-D35' showed the lowest drought tolerability. The effects of water stress on fruit chemical and biochemical traits varied significantly among cultivars, exhibiting an overall significant improvement in fruit quality. Two-dimensional clustered heatmap analysis subdivided the cultivars into two distinct clusters, mainly discriminated based on stability indexes of SSC, MI, TPC and TAC. Among the latter, SSC stability index was probably the most significant drought tolerance marker for Japanese plum.

1. Introduction

Plums are among the most widespread deciduous fruit crops, fairly varied in terms of species and varieties, both wild and cultivated, grown over a wide range of soils under different climatic conditions (Sudar et al., 2011). The genetic variability within plum species is remarkable and has a great influence on their production and consumption worldwide. The most commercially produced species are either Japanese plum (*Prunus salicina* L., 2n = 16), almost exclusively used for fresh consumption, or European plum (*Prunus domestica* L., 2n = 48) generally used for processing (Butac et al., 2019). Today, China (6.7 million tons), Romania (513 000 tons), Serbia (463 000 tons) and the United States (392 000 tons) are the main producers of commercially grown plums worldwide. With an overall production of 205 000 tons, Morocco is the ninth plums producer worldwide and the second in the Mediterranean area after Turkey (297 000 tons) (FAO, 2020). The Moroccan plum orchards are mainly dominated by Japanese varieties, especially 'Golden Japan', 'Santa Rosa', 'Formosa', 'Methley', 'Red Beauty', 'Angeleno' and 'Black Amber'. Local seedling cultivars are also grown in some areas, the most known of which are 'Zerhouni', 'Fassi', 'Meless' and 'Zuitni'. European varieties are once little cultivated, but have an increasing planting area due to the development of processing infrastructures and facilities, notably for the varieties 'Stanley' and 'Prune d'Ente' (Oukabli and Mamouni, 2005).

Plum producers' incomes depend mainly on yield, which is usually determined by tree load and fruit weight. Plum quality is also an important component of the fruit marketability, both for fresh consumption and industrial processing. Among global producing regions, plum requires irrigation to maximize yield and optimize fruit quality. However, in dry years, water resources may be insufficient to optimize irrigation and to obtain the maximum yield and thus optimum incomes level (Lopez et al., 2010). Under water stress, decreased yield is due to a reduction in fruit weight at harvest, or pre-harvest fruit drop or even both (Lopez et al., 2012). Fruit growth could be limited by two mechanisms: (i) direct limitation of fruit growth owing to a reduction in cell division and turgor in response to water stress (Le Gall et al., 2015), and (ii)

* Corresponding author.
E-mail address: razouk@yahoo.fr (R. Razouk).

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indirect limitation by a decrease in photosynthetic rate, as a result of stomata closure and a decrease in nutrients availability (Grappadelli and Lakso, 2004). Reductions in yield and fruit weight under drought conditions have been previously reported for plum, of which the magnitude was dependent to water stress level along with the phenological stage during which the water deficit was applied (Battilani, 2004; Intrigliolo and Castel, 2010; Ruiz-Sanchez et al., 2010). Other factors driving the yield reduction magnitude induced by water stress were the cultivar and the rootstock used, as previously reported for 'Red Beauty' cultivar grafted on 'Mariana 2624' rootstock and 'Black Gold' grafted on 'Mariana GF81' (Intrigliolo and Castel, 2010; Samperio et al., 2015).

Water stress can modify fruit chemical traits (soluble solids concentration, titratable acidity, pH) and biochemical compounds such as soluble sugars, amino acids, polyphenols and tannins (Wu et al., 2002; Falchi et al., 2020). Fruits from water-stressed plum trees had in general higher concentration of soluble solids and phenolic compounds than those from well-irrigated trees (Naor et al., 2004). As for water stress effect on fruit acidity, it was not as significant as that reported for sugars (Battilani, 2004; Naor et al., 2004), and only few works have reported a decreasing pattern as a response to water stress (Maatallah et al., 2015; Razouk et al., 2021).

Research aimed at improving yields and fruit quality under drought conditions are mainly based on two approaches: i) optimization of deficit irrigation by testing fractions of crop water requirements over different phenological stages, and ii) investigation of the intraspecific variability to select drought tolerant cultivars (Galindo et al., 2018; Adiba et al., 2021). Today, few works have been interested in screening the water stress effect on plum tree performance, leading to identify potential drought tolerant cultivars (Bojkova et al., 1998; Paudel et al., 2020). Searching for drought tolerant cultivars is certainly a strategic goal in plum breeding programs. It must first take into account the phenotypic variability of external factors, including yield and fruit quality, which determine the commercial value of the cultivar and thus its adoption degree by farmers. Such studies are much more justified in areas where climate change scenarios are alarming, which is the case for the Mediterranean basin, especially in southern countries. So far, none of the cultivated plum varieties are known to be worldwide more tolerant to water stress. In addition, knowledge in terms of discriminating selection criteria for drought tolerance in plum remains to be deepened, which will be of great use in breeding programs and thus in fostering the species cultivation in marginalized areas.

In this context, the present work aims to screen the plum phenotypic plasticity to drought through yield along with fruit weight and biochemical traits across eleven Japanese plum cultivars conducted under water deficit conditions. A particular interest was given to investigate correlations between all aforementioned variables and factors as a way to deepen our understanding of drought tolerance mechanism as well to determine the most significant drought markers. This study is therefore an important step to select drought-tolerant plum cultivars, which constitutes, to the best of our knowledge, the first research on this topic over cultivars grown in Morocco.

2. Materials and methods

2.1. Plant material and experimental conditions

This study was carried out during two consecutive years (2019–2020) at the experimental station of the National Agricultural Research Institute at Ain Taoujdate, northern Morocco (latitude 33°56′N, -5°13′O, 499 m asl). The climate of the research area is semiarid, characterized by low annual rainfall and long period of drought occurring frequently between March and September. The soil in the experimental plot is sandy-clay textured, moderately alkaline (pH = 7.68), slightly calcareous (3.05% $CaCO_3$) and moderately rich in organic matter (2.04%).

Eleven early ripening Japanese plum cultivars of similar age (twelve-year-old) and vigor were involved in this study. The trunk cross sectional area was indeed similar averaging 290 cm^2, with the same number of main branches for all trees (4 branches per tree). Two cultivars were exotic varieties, 'Santa Rosa' (harvested in June 21) and 'Goldan Japan' (June 24). The remaining nine are local seedling cultivars, preselected in northern Morocco: 'Timhdit' (June 19), 'Rosa-34' (June 21), 'Red-B38' (July 2), 'Black-A41' (July 2), 'Black-D35' (July 8), 'Black-G40' (July 8), 'Obil-42' (July 8), 'Fortu-43' (July 8) and 'Black-S46' (July 2). All cultivars were grafted on the 'Myrobolan' rootstock and planted following a randomized complete block design with 10 replicates each and spaced 5 × 5 m. All trees were pruned similarly and received the same fertilization, namely 150-90-180 g $tree^{-1}$ of N–P–K, determined according to soil analysis in dormancy period. In addition, a hand fruit thinning was applied at fruit set stage, before applying water treatments in order to homogenize the tree fruit load for all cultivars.

2.2. Irrigation treatments

In each year of the experiment, irrigation started in February (flowering stage) for all cultivars by drip system, based on daily crop water requirements (ETc), using two emitters per tree delivering 16 L h^{-1} each. Since fruit set stage in late March, the emitters have been changed in five neighboring trees of each cultivar by other emitters delivering 8 L h^{-1} to give two water treatments until leaf fall in autumn (end October): control irrigation of 100% ETc (CI) and a deficit irrigation regime of 50% ETc (DI), each applied on five trees. During the rainy periods, it was taken into account the effective rainfall values, equivalent to 80% of the recorded rainfall by the weather station, located at less than 1 km of the experimental orchard. In order to avoid interactions in soil water use between adjacent trees of different cultivars, each water treatment was applied on a separate block containing all cultivars. For each cultivar, three central trees per treatment were chosen for measurements, whereas, the other trees acted as buffer plants.

Crop water requirement was daily scheduled according to the ET_0 values and the crop coefficients (Kc) obtained by Doorenbos and Pruitt (1977). The Kc values were adjusted to tree canopy cover using a reduction coefficient of 0.94, which was similar for all trees as they have comparable vigor, calculated according to the formula of Fereres et al. (1981). The monthly amounts of water applied for each treatment are presented in Table 1.

2.3. Measurements

At harvest of each cultivar, 90 mature fruits per treatment (30 fruits per replicate) were sampled from 10 randomly selected fruiting branches for mean fruit weight determination using a precision balance 0.001 g, as well as for chemical and biochemical analysis. This fruit sampling method was adopted because it takes into account the variability in fruit size within individual trees. After fruit sampling, the remaining fruit tree load was weighed in the field to determine total fruit yield.

Fruit chemical quality measurements included titratable acidity (TA), pH, and total soluble solids (TSS). TA was determined over a sample of 5 g of pulp following the method of Lichou (1998). The pH was measured directly on freshly extracted juice through crushing the pulp. TSS expressed as degrees Brix were measured on drops of the same juice above using a refractometer (Atago PAL-1, Tokyo, Japan).

Fruit biochemical analysis included total contents of soluble sugars, amino acids and phenolic compounds as well as total antioxidant capacity. Soluble sugars and amino acids were extracted according to the method of Babu et al. (2002) on 5 g of pulp ground in 10 mL of 80% ethanol, and concentrations were determined by spectrophotometry following the method of Dubois et al. (1956) for sugars, Yemm and Cooking (1955) for amino acids. Phenolic compounds were extracted by grinding 5 g of pulp in concentrated methanol and analyzed following the Folin-Ciocalteu method, as described by Singleton and Rossi (1965). Total antioxidant capacity was measured on ethanolic extract from pulp

Table 1. Monthly cumulative values of reference evapotranspiration, effective rainfall and amounts of applied water during the two years of the experiment.

	Kc	ET_0 (mm)		Effective rainfall (mm)		Applied water (m^3 $tree^{-1}$)			
		2019	2020	2019	2020	CI_{2019}	DI_{2019}	CI_{2020}	DI_{2020}
Mar	0.85	78	79	17	46	0.68	0.68	0.23	0.23
Apr	0.95	91	89	25	44	0.85	0.24	0.53	0.00
May	1.05	141	128	0	24	2.09	1.04	1.53	0.58
Jun	1.15	142	158	2	2	2.28	1.13	2.53	1.25
Jul	1.15	146	176	0	10	2.36	1.18	2.72	1.29
Aug	1.15	140	145	0	8	2.26	1.13	2.23	1.06
Sep	1.10	107	112	2	4	1.62	0.79	1.67	0.80
Oct	0.90	64	62	11	14	0.65	0.24	0.57	0.18
Total	-	955	1004	84	157	12.79	6.43	12.01	5.39

Kc: crop coefficient; ET_0: reference evapotranspiration; CI: control treatment; DI: deficit irrigation.

samples, following the phenyl-1-picrylhydrazyl (DPPH) assay, based on the method of Brand-Williams et al. (1995).

2.4. Statistical analysis

SPSS v22 was used to perform data analysis. After testing normality of the data, the analysis of variance (ANOVA) was performed to test significant differences between irrigation treatments and Student-Newman and Keuls test (SNK) was done to compare sample means between the cultivars under each irrigation treatment. For each cultivar, the results were presented as sample means under each water treatment and also as trait stability indexes (TSI), expressed as mean ratios of DI to CI treatment. Pearson correlation test was applied on TSI values to identify the relationships between water stress effects on the observed traits. Principal component analysis was performed using correlation matrix on TSI standardized values using logarithmic function so as to have comparable scales. Likewise, two-dimensional clustered heatmap was performed on TSI values to classify cultivars based on their response to drought and that using Euclidean distance, while the variables were clustered using correlation distance.

3. Results and discussion

3.1. Yield and fruit weight

Significant differences in yield and fruit weight were observed between the studied cultivars in response to DI treatment. During the two-year field experiment under water deficit conditions, yield was reduced in 10 cultivars, while in 'Black-A41', yield decrease was only exhibited during the second year (Table 2). Compared to CI treatment, yield decrease rate ranged from 21% for 'Fortu-43' to 95% for 'Timhdit' in 2019, while in 2020, this decrease was of 8% for 'Golden Japan' and 78% for 'Black-D35'. The obtained yield in stressed trees varied from 3.72 kg (Obil-42) to 88.63 kg (Fortu-43) in 2019 and from 2.89 kg (Timhdit) to 46.37 kg (Rosa-34) in 2020. According to average water use efficiency (WUE) for the two years of the experiment, the most water efficient cultivar was 'Fortu-43' (WUE of 10.68 kg m^{-3} per tree), while the less efficient seemed to be 'Timhdit' and 'Obil-42', with WUE values less than 1 kg m^{-3}. Fruit weight diminished in all cultivars as a response to DI treatment, although in some cultivars this pattern was significant in only one year, which is the case for the cultivars 'Black-G40', 'Black-A41' and 'Fortu-43' (Table 3). Except the latter, the decrease rate in fruit weight in the other cultivars was of 5–24% in 2019 and in the range of 15–62% in 2020.

The literature reveals a significant decrease of yield and fruit weight in plum as a response to water deficit, particularly when applied at severe level. However, the magnitude effect was dependent to cultivars, which would be related to a genotypic difference in plum plasticity to drought (Maatallah et al., 2015). In contrast, some previous studies have reported a significant plasticity to drought, through maintaining normal yield level and fruit weight even under water deficit of 50% ETc, as observed in 'Angeleno' and 'Red beauty' (Scott Johnson et al., 1994; Moniño et al., 2020). Plum cultivars tolerance to drought depends also on the environmental conditions under which they were grown. For example, yield

Table 2. Yield (kg $tree^{-1}$) and water use efficiency (kg m^{-3}) of the studied plum cultivars in response to deficit irrigation.

	$Yield_{2019}$			$Yield_{2020}$			WUE_{DI}		
	CI	DI	YSI	CI	DI	YSI	2019	2020	Mean
Golden Japan	40.57 ab	27.18 c	0.67*	10.51 b	9.66 ab	0.92*	5.53 c	2.28 ab	3.91 ab
Santa Rosa	71.82 b	28.01 c	0.39**	45.92 e	30.30 d	0.66**	5.83 c	7.20 d	6.52 b
Timhdit	97.34 bc	4.87 a	0.05**	4.02 a	2.89 a	0.72**	1.03 a	0.70 a	0.87 a
Rosa-34	23.69 a	12.08 b	0.51*	57.97 f	46.37 e	0.80*	2.52 b	11.01 c	6.77 b
Black-D35	42.05 ab	21.03 c	0.50*	14.80 bc	3.25 a	0.22**	3.84 c	0.72 a	2.28 ab
Red-B38	81.90 b	52.42 cd	0.64*	9.63 b	7.70 ab	0.80*	10.02 cd	1.83 ab	5.92 b
Black-G40	79.42 b	12.71 b	0.16**	23.97 c	18.21 bc	0.76*	2.32 b	4.03 bc	3.18 ab
Black-A41	36.85 ab	36.11 c	0.98	29.76 de	20.53 bc	0.69**	6.91 c	4.87 bc	5.89 b
Obil-42	31.00 ab	3.72 a	0.12**	5.70 ab	5.07 ab	0.89*	0.68 a	1.12 ab	0.90 a
Fortu-43	112.19 c	88.63 d	0.79*	27.11 d	23.31 c	0.86*	16.20 d	5.16 c	10.68 c
Black-S46	46.44 ab	26.94 c	0.58*	10.15 b	6.59 ab	0.65**	5.15 c	1.56 ab	3.36 ab
ANOVA p-value	0.025	0.006		0.009	0.007		0.006	0.008	0.011

CI: control treatment; DI: deficit irrigation; YSI: yield stability index (yield DI/yield CI); WUE_{DI}: water use efficiency under DI treatment.
Within columns of CI, DI and WUE. marked values with different letters are significantly different at $P \leq 0.05$ according to SNK test.
Marked TSI values are significantly different to 1 at $P \leq 0.05$ (*) or $P \leq 0.01$ (**) by ANOVA.

Table 3. Fruit weight (g) under the CI and DI treatments and its stability index values in the studied plum cultivars.

	2019			2020		
	CI	DI	FWSI	CI	DI	FWSI
Golden japan	56.36 b	43.40 ab	0.77*	32.76 ab	19.88 a	0.61**
Santa Rosa	28.91 a	21.97 a	0.76**	24.30 a	17.07 a	0.70**
Timhdit	43.62 b	39.69 ab	0.91*	40.00 b	28.75 bc	0.72*
Rosa-34	28.74 a	22.42 a	0.78**	28.12 a	23.91 ab	0.85**
Black-D35	85.91 c	73.02 bc	0.85**	60.12 c	41.83 c	0.70**
Red-B38	75.85 bc	72.06 bc	0.95**	58.70 c	42.08 c	0.72*
Black-G40	79.94 bc	78.34 c	0.98	86.58 d	32.90 bc	0.38**
Black-A41	66.64 b	59.98 b	0.90*	40.30 b	40.10 c	0.99
Obil-42	72.50 bc	60.90 b	0.84**	41.38 b	24.85 ab	0.60**
Fortu-43	74.20 bc	65.30 bc	0.88	52.25 bc	26.77 bc	0.51**
Black-S46	89.62 c	78.87 c	0.88*	63.32 c	49.23 d	0.78*
ANOVA p-value	0.036	0.016		0.003	0.003	

CI: control treatment; DI: deficit irrigation; FWSI: fruit weight stability index (weight DI/weight CI).
Within columns of CI and DI. marked values with different letters are significantly different at $P \leq 0.05$ according to SNK test.
Marked TSI values are significantly different to 1 at $P \leq 0.05$ (*) or $P \leq 0.01$ (**) by ANOVA.

and fruit weight of 'Santa Rosa' variety were not significantly affected by water deficit of 50% ETc in an experiment carried out in Iran, contrary to results reported for a similar trial conducted in Turkey (Khandani et al., 2019; Yildirim, 2008). These contradictory results would be linked to differences in terms of the used rootstocks and agricultural practices, especially mineral nutrition management (Lopez et al., 2012; Dekena et al., 2017). Based on yield and fruit weight stability indexes, 'Santa Rosa' was shown to be more sensitive to water stress than 'Golden Japan', using 'Morybolan' as rootstock. In addition, the two local cultivars 'Black-A41' and 'Fortu-43' displayed a significant tolerance level, whereas, 'Timhdit' and 'Black-D35' stood out as the most sensitive.

In most cultivars, decrease rate of yield in response to DI treatment was generally higher than those of mean fruit weight, as indicated by comparing the TSI values. This suggests that the reduced yields under DI treatment originated especially from fruit drop, which were generally combined to a significant reduction in fruit weight. However, it was noted in some cultivars a decrease in yield due to fruit drop only, in absence of significant water stress effect on fruit weight, as observed in 'Black-G40' and 'Fortu-43' in the first year. In fact, fruit drop and production of small fruits are obvious reactions in various plants subjected to water deficit conditions, which are mainly linked to a significant decrease in transpiration and photosynthesis (Gindaba et al., 2004; Farooq et al., 2009).

On the other hand, all the studied cultivars showed yield alternation under both full and deficit irrigation. However, its intensity was more marked under DI treatment, with an average difference in yield of 45% between the two years of the experiment, against 32% under CI treatment. This phenomenon seems to be more accentuated by water stress on certain cultivars such as 'Timhdit' and 'Obil-42' compared to others such as 'Fortu-43', 'Black-S46' and 'Red-B38'. It is mainly attributed to competition for assimilates during floral induction and fruit growth, which becomes more intense under water stress conditions (Goldschmidt and Sadka, 2021). However, it should be noted that monitoring the behavior of cultivars under water stress for several years is necessary for a more conclusive assessment of water stress effect on yield alternation.

3.2. Fruit chemical traits

Since the first year of the experiment, the plum cultivars showed significant differences in fruit chemical proprieties as a response to water deficit (Table 4). TSS was significantly increased by DI treatment in six cultivars by averages of 1.30–4.83 °Brix, compared to control trees. Among these cultivars, 'Black-A41' and 'Obil-42' showed an increase in TSS in the first year, while in the second year, similar pattern was particularly observed in 'Timhdit', 'Black-D35' and 'Fortu-43'. On the other hand and in both year experiment, 'Black-S46' exhibited a significant rise of TSS content. However, in the other cultivars, TSS remained unaffected by water deficit. The increase of TSS values of the rosaceous fruits in response to water stress has been reported in several similar studies (Kobashi et al., 2000; Thakur and Zora, 2012). It has generally been linked to an induction of a higher starch hydrolysis and carbohydrates translocation in favor of fruit growth (Genard et al., 2003), as well as to a decrease in fruit water content, thereby increasing concentration of sugars in fruit cells (Cheng et al., 2003). In addition, the increase in TSS in different plant organs, including fruit, constitutes a drought tolerance mechanism by participating in maintaining, as high as possible, the turgor and the cytological volume of cells (Stefanelli et al., 2010). Soluble solids also allow preservation of membrane integrity in desiccated organs as well as protection of proteins (Hamann et al., 2015). However, some studies reported that under water stress, the increase in TSS may be not significant during the two first years, as observed in peach by Zhou et al. (2017). This observation may indeed explain the non-significant changes in TSS between CI and DI treatments in some cultivars, thereby suggesting variability in expressed genes involved in sugars metabolism within the studied plum collection in response to water stress (Liu et al., 2020).

As for TA, it was diminished by DI treatment in all cultivars compared to control group. The decrease in TA was significant during only the first year of the experiment and that for some cultivars, as observed in 'Golden Japan' in 2019 with a rate of 21%, as well as in 'Red-B38', 'Rosa-34' and 'Obil-42' in 2020 following the average rates of 6%, 14% and 21%, respectively. However, TA displayed a decreasing trend during both years for other cultivars following rates ranging from 10% (Fortu-43) to 33% (Black-S46). Previous studies have reported controversial results regarding the effect of water stress on TA in plum, which varied depending on the genetic factor and water stress application stages (Crisosto et al., 2004; Intrigliolo et al., 2010). Usually, a significant decrease in TA is observed when the water stress is applied over a long period, which is in agreement with our results (Maatallah et al., 2015; Hajian et al., 2020). With except of 'Black-D35', a slight significant increase in fruit pH values was observed in all cultivars in response to water stress. This increase occurred during the two consecutive years in the six cultivars, 'Golden Japan', 'Timhdit', 'Red-B38', 'Black-G40', 'Black-A41' and 'Black-S46', with an average pH rise of 0.18–0.71, while it was only observed in the second year in 'Santa Rosa', 'Rosa-34' and 'Fortu-43', in which the respective pH increase values were of 1.27, 0.13 and 0.14. During the first year of the experiment, this increase was only displayed by 'Obil-42', with an average of 0.39. The effects of drought over the fruit pH were previously reported by Razouk et al. (2013), who observed that plum pH of 'Stanley' variety (European plum) remained unchanged under deficit irrigation over three consecutive growing seasons. Contrary, Maatallah et al. (2015) reported a significant increase in fruit pH in three Japanese plums, 'Black Gold', 'Black Diamond' and 'Black Star' in response to water deficit. Therefore, the increase in fruit pH under water stress may be significant or not depending on genotypes, although it seems common on plum, since most studied cultivars displayed an increasing trend as response to water stress.

According to the aforementioned changes over TSS and TA, all cultivars showed a significant increase in maturity index (MI), except for 'Rosa-34'. For 'Timhdit', 'Red-B38', 'Black-A41' and 'Obil-42', MI increased in the second year of DI treatment application (2020). However, in the other cultivars, this effect was recorded since the first year (2019), having persisted in the second year (2020). Over the two consecutive years of the experiment, the highest increase rate of MI in response to DI treatment was showed by 'Black-D35', which averaged 53%, while the lowest was of 9%, observed in 'Red-B38'. The two exotic varieties, 'Santa Rosa' and 'Golden Japan' showed an increase rate of MI

Table 4. Stability indexes of total soluble solids (TSS). titratable acidity (TA). pH and maturity index (MI) in fruit pulp of the studied cultivars in response to DI treatment.

	TSS index		TA index		pH index		MI index	
	2019	2020	2019	2020	2019	2020	2019	2020
Golden Japan	1.04	1.05	0.79**	1.05	1.05*	1.03*	1.16*	1.32**
Santa Rosa	1.01	1.03	0.86**	0.84**	1.01	1.35**	1.14**	1.14*
Timhdit	1.00	1.32**	0.85*	0.85*	1.07*	1.20*	1.02	1.67**
Rosa-34	1.04	1.05	0.99	0.86*	1.01	1.03*	1.04	1.00
Black-D35	1.09	1.15**	0.64**	0.73**	1.02	1.01	1.69**	1.36**
Red-B38	1.04	1.01	0.98	0.94*	1.04*	1.11*	1.02	1.18**
Black-G40	1.01	1.00	0.89*	0.89*	1.09*	1.05*	1.12*	1.15*
Black-A41	1.16**	1.05	0.83**	0.85*	1.02*	1.27**	1.03	1.43**
Obil-42	1.17**	1.05	0.84	0.79**	1.09*	1.02	1.02	1.24**
Fortu-43	1.01	1.14**	0.89*	0.90*	1.02	1.03*	1.13*	1.27**
Black-S46	1.14**	1.11**	0.54**	0.79**	1.12*	1.19*	1.64**	1.30**

Marked values are significantly different to 1 at $P \leq 0.05$ (*) or $P \leq 0.01$ (**) by ANOVA.

of 14% and 24%, respectively, compared to CI treatment. Therefore, DI improved fruit sweetness in all cultivars, which seems important to increase their acceptance by consumers (Iglesias and Echeverria, 2009). In fact, fruit from stressed trees looks like that of those in advanced maturity stage, since changes in TSS, TA and pH follow their normal trend during the fruit ripening stage, even under full irrigation. Some studies have linked changes in rosaceous fruits taste under water stress to their early maturity, due to an increase in solar interception owing to a decrease in vegetative growth, making the fruits ripen faster (Lopez et al., 2008; Galindo et al., 2017).

3.3. Fruit biochemical traits

Results of DI effect on biochemical traits are summarized in Table 5. Indeed, DI treatment significantly increased total soluble sugar content (SSC) in all cultivars. In nine cultivars, the increase of SSC occurred in both years of the experiment, by an overall average of 35% in 2019, against 27% in 2020. The increase in SSC was therefore generally higher in the first year in this group of cultivars, although some of them showed a greater increase in the second year. Thus, the highest increase of SSC was of 76% shown by 'Timhdit' in 2020, while the lowest value was of 4%, observed in 'Obil-42' in 2019. Particularly for 'Black-A41', the increase of SSC was only significant in 2019 by a rate of 26%, and in 2020 for 'Santa Rosa' by 12%.

Similar results have been reported in several previous studies on various rosaceous fruits (Kobashi et al., 2000; Cheng et al., 2003; Thakur and Zora, 2012). In case of plum, it has been demonstrated that the increase in SSC is mainly related to an increase in sucrose content. However, the contents of glucose and fructose were shown to be rather reduced under water deficit conditions (Maatallah et al., 2015; Haider et al., 2018). Increased sucrose content under water stress was related to an induction of rapid conversion of starch reserves (Genard et al., 2003; Becel et al., 2010), a fact which could also be attributed to an inhibition of starch synthesis (Guichard, 2005; Miras-Avalos et al., 2013). This suggests that accumulation of soluble sugars in fruit under water deficit conditions, results mainly from a metabolic imbalance of sugars in fruit cells. In addition, some reports have indicated that the accumulation of sucrose in cells can increase drought tolerance in plants by limiting water loss due to its osmotic effect (Tomasella et al., 2020). These observations would suggest a higher translocation or synthesis of sucrose in the studied plum cultivars in response to DI treatment. This may indeed improve fruit taste as well as drought tolerance plasticity, notably in cultivars that showed a higher increase in SSC under DI treatment in both years of the study, such as 'Golden Japan' (+61% in average) and 'Timhdit' (+46%).

Contrary to SSC, data showed a significant decrease of total amino acid content (AAC) in response to DI treatment, giving to fruit a sweeter taste. In 2019, AAC decreased by 10–63% in six cultivars, namely in ascending order 'Red-B38', 'Black-D35', 'Rosa-34', 'Timhdit', 'Santa Rosa' and 'Golden Japan'. However, in 2020, decreases in AAC were significant in all plum cultivars with a rate ranging from 8% in 'Timhdit' to 33% in 'Black-G40'.

Similar results were reported for cv. Stanley as well as for peach and almond (Lombardo et al., 2011; Razouk et al., 2021). In fact, it is

Table 5. Stability indexes of soluble sugars content (SSC). amino acids content (AAC). total phenolic compounds (TPC) and total antioxidant capacity (TAC) in fruit pulp of the studied cultivars in response to DI treatment.

	SSC index		AAC index		TPC index		TAC index	
	2019	2020	2019	2020	2019	2020	2019	2020
Golden Japan	1.47**	1.24*	0.76*	0.73*	1.06	1.08	1.04	1.12
Santa Rosa	1.01	1.12*	0.84*	0.72*	1.09	1.04	1.14*	1.07
Timhdit	1.46**	1.76**	0.90*	0.92*	1.13*	1.29**	1.03	1.15*
Rosa-34	1.45**	1.16*	0.94	0.72*	1.65**	1.36**	1.26*	1.07
Black-D35	1.35**	1.22*	0.37**	0.80*	1.13*	1.10*	1.15*	1.10
Red-B38	1.13*	1.16*	0.88*	0.77*	1.14*	1.03*	1.05	1.33**
Black-G40	1.46*	1.20*	0.74	0.67**	1.43*	1.47**	1.22*	1.78**
Black-A41	1.26**	1.03	0.65	0.72*	1.02	1.01	1.63*	1.37**
Obil-42	1.04*	1.27**	0.82	0.76*	1.28*	1.75**	1.02	1.26**
Fortu-43	1.54**	1.37**	0.56	0.77*	1.22*	1.11*	1.07	1.29**
Black-S46	1.28**	1.21*	0.76*	0.80*	1.29*	1.07	1.02	1.76**

Marked values are significantly different to 1 at $P \leq 0.05$ (*) or $P \leq 0.01$ (**) by ANOVA.

generally agreed that fruit tree species tend to have lower AAC in their fruits under water deficit conditions, although the involved mechanisms remain unclear. However, three main hypotheses have often been advanced to explain this water stress effect, i) the use of amino acids as precursors of sugar synthesis, ii) the decrease in their biosynthesis at leaf level due to low absorption of nitrogen under water deficit, and iii) the partial inactivation of their synthesis in fruit due to a decrease in content of reducing sugars, especially glucose, being the main origin of the carbon skeleton of amino acids (Zushi et al., 2006; Ogasanovic, 2007).

Total phenolic content (TPC) displayed different response patterns to DI treatment. Hence, TPC was not affected in 'Golden Japan', 'Santa Rosa' and Black-A41. In Red-B38 and Black-S46, DI treatment only increased TPC in the first year (2019), with the rates of 14% and 29%, respectively. For other cultivars this pattern was observed during the two consecutive years of the study, of which the average rates were in the range of 12% in 'Black-D35' and 52% in 'Obil-42'. Similarly, Maatallah et al. (2015) observed that TPC was significantly increased in 'Black Star' and 'Black Diamond' by deficit irrigation of 50% ETc, while it was not affected in 'Black Gold'. The total amount of phenolic compounds and their profiles are known to be sensitive to water stress, due to the expression of structural and regulatory genes involved in their biosynthesis pathway, with different amplitudes according to the genotypes (Kumar et al., 2020). In addition, Gelly et al. (2004) reported that changes in TPC under water stress are related to higher exposure of fruit to sunlight due to a decrease in vegetative growth. This implies that these factors influencing phenolic compounds biosynthesis under water stress, either directly or indirectly, have therefore been favorable for an enhancement of the synthesis of the predominant phenolic compounds in certain cultivars, particularly chlorogenic acid, epicatechin and quercetin (Mubarak et al., 2012). In addition, in some others cultivars, water stress would have induced changes in the phenolic compounds profile without impacting their total content in fruit, as it may explain the tendency of TPC in 'Golden Japan', 'Santa Rosa' and 'Black-A41' as response to applied DI. Moreover, increasing TPC is known to be as positive response to water stress, improving the nutritional and dietetic fruit quality (Hassan et al., 2021), but also a drought-resistance mechanism via a contribution in the anti-oxidative defense system by trapping reactive oxygen species (Ripoll et al., 2014).

Total antioxidant capacity (TAC), using the DPPH method, was not affected by DI treatment in 'Golden Japan'. However, the other cultivars showed a significant increase in TAC under DI conditions compared to CI, where the average rate was in the range of 9–50%. For 'Santa Rosa', 'Black-D35' and 'Rosa-34' the increase was only observed in 2019 with the following rate 15%, 37% and 76%, respectively. In 2020, 'Timhdit', 'Red-B38', 'Obil-42', 'Fortu-43' and 'Black-S46' displayed the same effect with rates ranging from 15% to 76%. This tendency was in fact anticipated, as it has often been linked to an increase in concentration of total antioxidant compounds, such as flavonoids and anthocyanins or to the emergence of certain phenolic compounds characterized by a higher antioxidant potential, such as quercetin in case of plums (Mubarak et al., 2012; Ripoll et al., 2014). In summary, these results confirmed the improvement in plum fruit quality under water stress, as reported in previous similar studies. However, the biochemical traits affected and the extent of their variation differed following the genotypic factor.

3.4. Correlation analysis

To understand the relationship between changes induced by water stress on the observed traits, a correlation test was performed using Pearson model on TSI means (Table 6). Such correlations are interesting to explain links between changes induced by water stress as well as to predict drought tolerance level in plum, based on a reduced number of traits. Indeed, the obtained correlation coefficients were significant between TSI values regarding fruit chemical traits only. They confirmed the known opposite relationship between TSS and TA evolution in fruits, often impacting their maturity index and which is commonly linked to the use of amino acids as precursors in sugar biosynthesis pathway (Lombardo et al., 2011). Regarding fruit biochemical traits, the correlation coefficients linking their TSI values were not statically significant. Although the correlation between SSC and TSS is known to be significant and positive, since sugars are the major soluble solids in fruit, it was found that this trend was not statistically significant. This pattern was previously reported mainly when evaluating a collection of various genotypes (Maatallah et al., 2015; Hssaini et al., 2019). This observation would be due to genotypic differences regarding potential richness in sugars and other soluble solids (organic and amino acids, soluble pectins, etc.). In addition, correlations between TPC and TAC in fruits have been variously reported in the literature as being positively or negatively correlated depending on the species and the experimental conditions (Mubarak et al., 2012; Miletic et al., 2012; Hssaini et al., 2020). Tlili et al. (2011) have even reported non-significant correlation between TPC and TAC in watermelon fruit. They attributed this result to the complexity of synergies and interactions between the various bioactive compounds involved in fruit antioxidant activity, including phenolic and non-phenolic molecules. In addition, Kristl et al. (2011) observed in four plum cultivars ('Valor', 'Stanley', 'Hanita' and 'Tophit') that non-extractable phenolic compounds contributed more than 80% to the TAC, suggesting that it may be underestimated using correlation with TPC. On the other hand, correlation between stability indexes of yield and fruit weight was not significant, indicating that decreases in yield, observed in most cultivars, were due to combined effects of fruit drop and fruit weight reduction. Furthermore, the non-significant correlation between TSI values of fruit weight and those of fruit chemical and biochemical traits would suggest that the observed changes on these last traits were not dependent on lower fruit growth under water deficit.

In a second correlation test, the relationships between the traits means under CI treatment and the TSI values were investigated to

Table 6. Matrix of correlation coefficients between mean values of traits stability indexes (TSI) of two experimental years.

TSI	FY	FW	TSS	PH	TA	MI	SSC	AAC	TPC	TAC
FY	1	0.205	-0.275	-0.083	0.452	-0.347	-0.163	-0.105	-0.349	0.197
FW		1	0.403	0.411	-0.178	0.17	-0.213	0.172	-0.318	-0.164
TSS			1	0.171	**-0.640***	**0.693***	0.375	0.056	-0.087	-0.094
PH				1	-0.236	0.158	-0.253	0.376	-0.385	-0.061
TA					1	**-0.838****	0.092	0.346	0.109	-0.088
MI						1	0.266	-0.349	-0.416	0.033
SSC							1	0.159	0.129	0.053
AAC								1	0.257	-0.281
TPC									1	0.155
TAC										1

TSI: trait stability index; FY: fruit yield; FW: fruit weight; TSS: total soluble solids; TA: titratable acidity; MI: maturity index; SSC: soluble sugars content; AAC: amino acids content; TPC: total phenolic compounds; TAC: total antioxidant capacity.
*, **: significant correlation coefficient at $p \leq 0.05$ or $p \leq 0.01$.

identify criteria that can predict certain changes induced by water stress (Table 7). This was the case of changes observed on maturity index, SSC and TAC, which seem related to the genetic potential of the cultivar under full irrigation in terms of yield, fruit weight, pH and SSC. Indeed, the obtained correlation coefficients indicated that i) the fruit maturity was highly increased by water stress in less productive cultivars (r = -0.620), ii) the TAC values were more increased in cultivars with higher fruit weight (r = 0.794), and iii) the ample increase in SSC values was observed in cultivars showing high levels in pH (r = 0.702) and SSC (r = 0.667). These four criteria may therefore be used as predictive indicators of changes in fruit quality in response to water stress. However, none of them predicted the stability indexes of yield and fruit weight in response to water stress, indicating the involvement of other factors at leaf or root levels (not herein assessed) in the plasticity degree of the studied cultivars to drought.

3.5. Multivariate analysis

To identify trait stability indexes that had the highest impact on discrimination among plum cultivars, principal component analysis (PCA) was performed using TSI means of two years of the experiment (Table 8). Indeed, about 50% of total variance was explained by the first two components, which were significantly correlated with TSI values of all traits, except fruit yield and TAC. The first component explained about 30% of total variance, which was strongly determined by stability indexes of TSS, TA and MI, with eigenvectors values higher than |0.8|. The second component accounted for about 20% of total inertia and was mainly correlated with TSI values of fruit weight (r = 0.676), pH (r = 0.650), SSC (r = -0.521) and TPC (r = -583). These results indicate that discrimination between cultivars in response to water stress was primarily linked to changes observed on chemical traits (TSS and TA). Changes in fruit weight, SSC and TPC are also discriminating variables between cultivars, but they are of secondary order.

Moreover, two-dimensional clustered heatmap built based on cultivars plasticity to water stress during the two consecutive experimental years, using TSI means, is presented in the Figure 1. It is an analytical representation form, widely used to visualize and analyze complex biological data by displaying network connections in a matrix of colors intensities, of which the lightest ones indicate strong correlations and vice versa (Clark and Ma'ayan, 2011). Based on the model, the TSI values of SSC, MI, TPC and TAC were the most important variables in the total variance explained, and thus captured the highest discrimination power. Differences between heatmap and PCA methods in identifying discriminant variables are related to the explained variance, which is total using heatmap analysis against PCA, since it does not require a dimensionality reduction. Nevertheless, both methods identified stability indexes of SSC and MI as potential discriminants variables. Two main clusters of cultivars are distinguished with respect to their response to water stress. The first main cluster (C1) includes three cultivars, 'Black-A41', 'Fortu-43' and 'Golden Japan', distinguished by a higher increase of fruit SSC in response to water stress. These cultivars' yields were the least affected by DI, which were diminished by respective average rates of 17, 18 and 21%. Hence, this cluster was classified as the most tolerant to drought within the studied plum collection, with a superiority of 'Fortu-43' cultivar owing to its higher WUE (10.68 kg m^{-3} per tree). The second main cluster (C2) is subdivided into two distinct sub-clusters. The first sub-cluster (C2-1) contains 'Santa Rosa', 'Timhdit', 'Black-S46' and 'Black-D35', showing a particular increase of MI under water stress, owing mainly to significant decrease of TA. This group is composed of two cultivars assessed as the most drought sensitive based their yield stability indexes, namely 'Timhdit' and 'Black-D35', which showed a lower WUE of 0.87 kg m^{-3} and 2.28 kg m^{-3}, respectively. The second sub-cluster (C2-2) is formed by 'Red-B38', 'Black-G40', 'Obil-42' and 'Rosa-34', where the increase of TPC and TAC tends to be higher under water stress. However, drought tolerance level of these cultivars was assessed as intermediate based on yield and fruit weight stability indexes,

Table 8. Eigenvectors of principal component (PC) of PCA based on trait stability indexes of the two experimental years.

TSI	PC1	PC2	PC3	PC4	PC5
Fruit yield	-0.398	0.480	-0.483	0.491	0.142
Fruit weight	0.397	**0.676**	0.108	0.153	0.172
TSS	**0.823**	-0.102	0.263	0.288	0.116
pH	0.370	**0.652**	0.250	-0.248	0.288
TA	**-0.880**	0.185	0.146	0.335	-0.080
MI	**0.930**	-0.187	-0.178	0.104	-0.085
SSC	0.142	**-0.521**	0.326	0.717	0.036
AAC	-0.197	0.290	**0.858**	0.088	0.217
TPC	-0.369	**-0.583**	0.428	-0.281	0.312
TAC	-0.065	-0.278	-0.480	0.025	**0.807**
Explained variance (%)	29.69	19.65	16.84	11.33	9.56
Cumulative variance (%)	29.66	49.34	66.18	77.52	87.08

TSI: trait stability index; TSS: total soluble solids; TA: titratable acidity; MI: maturity index; SSC: soluble sugars content; AAC: amino acids content; TPC: total phenolic compounds; TAC: total antioxidant capacity.
Significant eigenvectors. higher than |0.5| are marked in bold.

Table 7. Matrix of correlation coefficients between traits values under CI treatement and traits stability indexes (TSI) based on means of two experimental years.

TSI	Traits values under CI treatment									
	FY	FW	TSS	PH	TA	MI	SSC	AAC	TPC	TAC
FY	0.012	-0.237	-0.16	0.253	0.009	-0.105	-0.127	-0.129	-0.342	0.081
FW	0.25	-0.501	-0.488	-0.191	0.088	-0.379	-0.468	-0.561	-0.106	-0.346
TSS	-0.365	-0.091	0.029	0.568	0.521	-0.328	0.548	0.041	-0.018	0.177
PH	0.199	-0.318	-0.551	0.171	-0.078	-0.394	-0.474	-0.596	-0.041	-0.292
TA	-0.025	-0.145	-0.187	0.151	0.401	-0.386	-0.338	-0.307	-0.026	0.069
MI	**-0.620***	-0.018	-0.141	0.218	0.557	-0.474	0.274	-0.057	-0.271	-0.194
SSC	-0.457	-0.025	0.074	**0.702***	0.503	-0.266	**0.667***	0.128	-0.106	0.369
AAC	-0.549	-0.083	-0.07	0.594	0.323	-0.265	0.486	-0.064	-0.195	0.358
TPC	-0.105	0.106	0.217	-0.121	-0.151	0.332	0.53	0.506	-0.313	0.149
TAC	-0.255	**0.794****	-0.017	-0.195	-0.477	0.246	-0.133	-0.007	-0.134	-0.238

TSI: trait stability index; FY: fruit yield; FW: fruit weight; TSS: total soluble solids; TA: titratable acidity; MI: maturity index; SSC: soluble sugars content; AAC: amino acids content; TPC: total phenolic compounds; TAC: total antioxidant capacity.
*, **: significant correlation coefficient at $p \leq 0.05$ or $p \leq 0.01$.

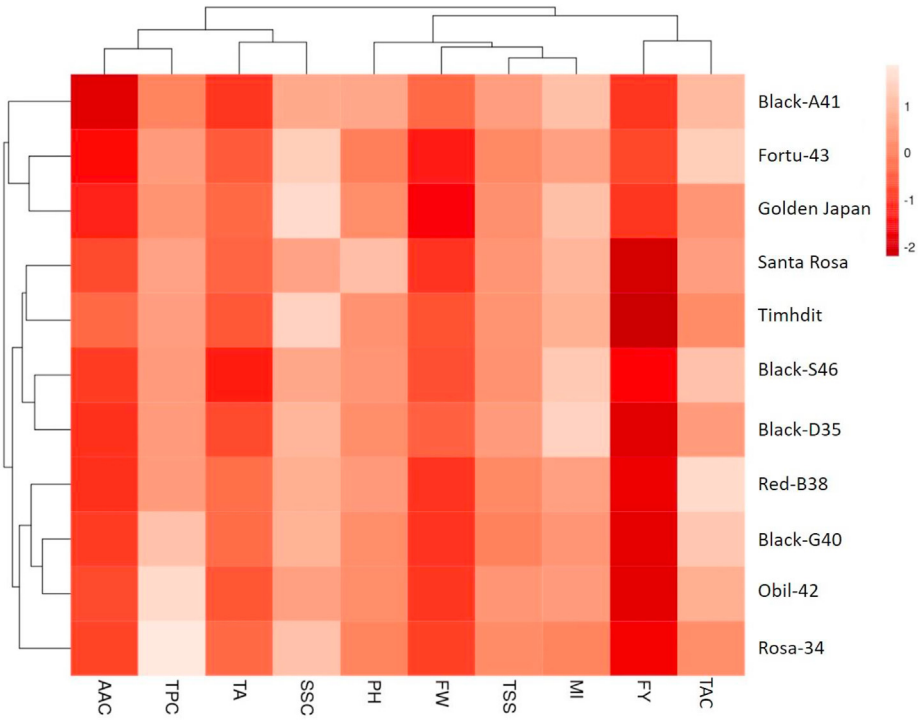

Figure 1. Hierarchically clustered heatmap based on the correlation matrix of traits stability indexes (averages of two years). The low color intensity means the highest TSI value and vice versa. FY: fruit yield; FW: fruit weight; TSS: total soluble solids; TA: titratable acidity; MI: maturity index; SSC: soluble sugars content; AAC: amino acids content; TPC: total phenolic compounds; TAC: total antioxidant capacity.

with a WUE average of 4.19 kg m^{-3}. However, it is important to emphasize that the clusters herein revealed may vary with the use of a different rootstock, other than 'Myrobolan' (Dekena et al., 2017).

Nevertheless, these results suggest that among the discriminating variables revealed by heatmap, fruit SSC would be a potential biochemical marker of drought tolerance in plum. Similar result was found by Jiménez et al. (2013), who reported that soluble sugars accumulation in leaves can also be used as phenotypic marker in assessing plum plasticity to water stress. However, fruit TPC would be rather a secondary marker, although it had a great impact on discrimination between cultivars in response to water stress. This multivariate analysis, carried out for the first time on a Moroccan plum collection, is therefore an important step to select drought-tolerant plum cultivars and to deepen the knowledge on the mechanisms involved.

3.6. Conclusion

Large differences were observed in behavior under water stress among the herein studied Japanese plums grafted on 'Myrobolan' rootstock. The differences in terms of yield, fruit weight and WUE under water stress were very significant, indicating a large phenotypic plasticity to drought within the studied collection. Among the stability indexes of fruit chemical and biochemical traits, those of soluble sugars, phenolic compounds, antioxidant capacity and maturity index were shown to be the most discriminating variables for cultivars plasticity level as a response to water stress. Soluble sugars stability index was distinguished as a potential marker of drought tolerance, since its values displayed an increasing pattern over the most efficient cultivars under water stress. Furthermore, the stability index of soluble sugars was positively correlated to their content under full irrigation, suggesting that this latter trait can also be considered as effective marker in screening plum drought tolerance. Further research will be needed to ascertain whether fruit chemical and biochemical traits participate in drought tolerance of plum cultivars and to unravel the soluble sugars specific-pathway changes in this metabolism. This study would be the basis for future investigations at physiological and molecular levels to in-depth investigate the mechanisms involved in plum drought tolerance.

Declarations

Author contribution statement

Anas Hamdani: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lahcen Hssaini: Analyzed and interpreted the data; Wrote the paper.

Said Bouda: Conceived and designed the experiments.

Atman Adiba: Contributed reagents, materials, analysis tools or data.

Rachid Razouk: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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