

**Changes of photosynthetic and antioxidant activity of *Phaseolus vulgaris* to
Potassium**

Running head: Potassium levels in common beans

ABSTRACT

Greenhouse experiment was conducted with two cultivars of common beans and four concentrations of potassium (1, 2, 10 and 20 millimolar –mM) were supplied to the soil pots aiming to access variations in plant antioxidant defense systems (secondary metabolites and enzymatic mechanisms), growth parameters and leaf gas-exchange measurements. The total fresh leaf mass increased in both cultivars from 1 to 10mM of potassium and the dry matter content decreased from 1 to 20mM. Root volume significantly increased in Uirapuru cultivar. Potassium increased chlorophyll A in both in the range of 1 to 10Mm. Catalase activity and carotenoids increased only in Dama cultivar. Potassium increased the photosynthetic activity in cultivar-dependent manner. Cultivar ‘Dama’ showed higher intrinsic water use efficiency, NDVI index, chlorophyll contents, total leaf mass and higher catalase activity. If the selection of cultivar under those traits is aimed, ‘Dama’ cultivar is a candidate and presented a linear stomatal conductance model.

Keywords: Common beans, Potassium nutrition, Photosynthesis, Stomatal conductance models, Growth parameters, antioxidant defense mechanisms, Phenolics, Normalized vegetative index, Catalase.

24 INTRODUCTION

25 Common bean (*Phaseolus vulgaris* L.) is the most important grain legume in human diets. It
26 provides protein, complex carbohydrates, and valuable micronutrients for more than 300
27 million people in the tropics. Is the second most important source of calories after maize in
28 Eastern and Southern Africa providing 32% of energy supply and the fourth in tropical America
29 where it is also the third most important source of calories after maize and cassava and
30 constitutes an important source of household income for small scale farmers [1, 2]. The
31 common beans are thought to provide up to 65% of protein [3] and currently estimated to be
32 one of the most important legumes worldwide [4,5]. Beside the great importance, there are
33 many factors that affect the high performance of the plant potential and photosynthesis.

34
35 Potassium (K^+) is the most abundant inorganic cation in plant cells. K^+ is vital for plant growth.
36 Together with nitrogen (N) and phosphorous (P), K^+ belongs to the top three elements, the
37 availability of which strongly determines crop yield. Undeniably, the application of mineral NPK-
38 fertilizers was an essential cornerstone of the green revolution in the last century [6]. Limited
39 resources [6] are now forcing us to investigate and better understand how the plants' K^+
40 demand can be satisfied with the minimum of fertilizer application [6]. The indispensable
41 ground for providing answers to this complex question is the understanding of the exact roles
42 of K^+ in plants [6]. Yao & Naeth [7] reported changes in physiological growth stages in barley
43 from emergence and below ground biomass in response to potassium application.

44
45 In plant metabolic processes, potassium application has been reported to inhibit the uptake of
46 cesium [8]. As reported by Rashid [9] potassium (K) has a critical role in plant physiology and
47 comprises up to 10% of plants dry weight [10]. K provides regulatory control over different

48 plant processes such as transpiration, starch synthesis, sucrose translocation, respiration and
49 lipid synthesis and has a profound effect on the profile and distribution of the primary
50 metabolites in plant tissues [9]. Changes in metabolite concentrations induced by K are multiple
51 and include K dependence of metabolic enzymes, photosynthesis and long distance transport
52 [9]. The primary metabolites such as soluble sugars particularly reducing sugars, organic acids
53 and amino acids tend to increase in K deficient plants [9]. Experiment conducted by Rashid [10]
54 found that K fertilization increased K content but reduced N, Si, free sugar and soluble protein
55 contents in the plant tissues which resulted in the minimum reduction of relative water content
56 (RWC) in rice plants.

57
58 Gautam and co-workers [11] also reported the beneficial effect of potassium application in
59 improving the submergence tolerance of rice. All the cultivars evaluated showed inhibition of
60 photosynthesis, and this was accompanied with decrease in stomatal conductance, chlorophyll
61 and carbohydrate contents. The activity of antioxidants was found to be significantly higher.
62 Potassium application improved the survival mainly because of maintenance of carbohydrates,
63 chlorophyll and contributing to less lodging and leaf senescence. Furthermore, K application
64 resulted in inhibition of lipid peroxidation and increase in catalase and peroxidase activities
65 [11]. Potassium at higher levels was more beneficial in terms of improving survival,
66 photosynthesis and plant growth [11]. Experiment conducted in peanut cultivars by
67 Chakraborty [12] showed that external K application resulted in improved salinity tolerance in
68 terms of plant water status, biomass produced under stress, osmotic adjustment and better
69 ionic balance. A study conducted by Zain & Ismail [13] in rice aiming to understand the effects
70 of potassium rates (80, 120 and 160 kg/ha) and types (KCl and K_2SO_4) on leaf gas exchange,
71 growth and biochemical changes under cyclic water stress concluded that increases in

72 fertilization rates increased proline production, catalase, malondialdehyde (MDA) and
73 transpiration rate. Higher potassium rates reduced water stress effects by inducing high
74 transpiration which increased nutrient uptake to repair the damaged tissues and reduce
75 oxidative stress. Potassium in KCl form increased net assimilation rate –NAR. Potassium has
76 been also reported to play different roles such as biophysical and biochemical roles, improve
77 tolerance to biotic and abiotic stress (salinity, drought, ammonium concentrations, cold, frost,
78 light), reduce oxidative stress load of chilling stressed plants, role in photosynthesis, movement
79 of plant organs, improve pathogen resistance, maintain osmotic potential of sieve tubes, cell
80 turgor and phloem loading and transport [14-17].

81
82 K constitutes about 2.1–2.3% of the earth's crust and thus is the seventh or eighth most
83 abundant element [14]. Therefore, soil K reserves are generally large [18]. However, large
84 agricultural areas of the world are reported to be deficient in K availability. Soils inherently low
85 in K are often sandy, waterlogged, saline, or acidic. Additionally, in intensive agricultural
86 production systems, K has become a limiting element, in particular in coarse-textured or
87 organic soils. In many cases, lower fertilizer K application in the context of unbalanced
88 fertilization may result in a significant depletion of available soil K reserves, and thus in
89 decreased soil fertility [14]. Beside extensive literature of potassium in other crops, a better
90 understanding on how potassium affect the photosynthetic and antioxidant is of crucial
91 importance to better manage the common beans.

92 Aiming to better understanding the role of potassium application in photosynthesis, agronomic
93 attributes, antioxidant behavior and secondary metabolism of common beans, greenhouse
94 experiment (pot experiment) was conducted with two contrasting cultivars (white-“carioca”
95 and black colored tegument)

96 **Materials and methods**

97 ***Plant material and soil preparation***

98 Two contrasting commercial common bean cultivars were selected for this experiment (white
99 colored grains-carioca “TAA DAMA”, hereinafter designed as Dama and black colored grains
100 “IPR88 UIRAPURU”, designed as Uirapuru, see Figure 1E). Dama cultivar is tolerant to rains even
101 during harvesting time; it has growth cycle of 85 to 95 days, with plant height around 50 cm. Its
102 growth habit is indeterminate (type III) and the shape of the grain is oblong. It has been argued
103 to be moderately resistant to rust, angular stain, mildew and bacteriosis and resistant to
104 common mosaic. Has long duration of light grain color for more than one year; higher
105 productive ceiling, extensive adaptation and higher sieve yield [19]. Uirapuru was selected
106 because presents excellent culinary qualities, is moderately tolerant to water stress [20], has a
107 broad adaptation, , with erect stems and branches which favors direct mechanical harvesting.
108 Flowers and reaches maturity at 43 and 86 days after germination respectively [21]. Resistant
109 to common mosaic virus, rust and powdery mildew and tolerant to high temperatures [21].
110 Sandy soil in the pots was firstly washed during 03 times (24 hours each) with distilled-
111 deionized water in other to remove any existence of minerals before seeding.

112

113 ***Greenhouse experiment and treatments***

114 The experiment followed a randomized block design, with three blocks. Each block consisted of
115 10 treatments (2 genotypes –“Dama” and “Uirapuru”, hereinafter designed as “UIRA” and four
116 levels of potassium- 0, 1, 2, 10 and 20 mM applied in a form of potassium chloride (KCl),
117 molecular weight of $74.548 \text{ g mol}^{-1}$ and the KCl mass required for each concentration calculated
118 according to the equation below –Eq. A). These 4 KCl concentration were chosen taking into
119 account the previously published manuscript by Nieves-Cordones [15] which state levels of

(>10mM) as higher concentration for uptake in non-selective channels (main pathways for K⁺ uptake) while 1mM as intermediate level[10]. So according to previous reports [10], 2 high and 2 intermediate levels were chosen and considered adequate for pot experiments. Treatments in each block were represented by 3 pots (previously filled with soil) with 3 plants in each in a total of 9 plants per treatment/block. Bean seeds were surface sterilized by a commercial fungicide (carbendazim + tiram at a dose 300 mL/100kg of seeds) before seeding in the pots and then 5 seeds were seeded and placed in the greenhouse under partially controlled conditions of light intensity, photoperiod, relative humidity and temperature. Seedlings were irrigated by basic salt of Murashige and Skoog nutrient solution (2mM potassium-total concentration and applied 1/4 per week) one time per week, during one month. Plants were watered regularly (one to two times per week) with distilled water and 30 days after potassium treatment, the evaluations were started.

$$m = [C] * M_w * V \quad (\text{Eq. A})$$

Where “m” is the required mass of KCl; [C] is the required concentration; Mw is the molecular weight of KCl and V is the total volume of water used (3.6 L).

Chlorophyll content

Chlorophyll (Chla, Chlb and total) in the greenhouse was determined using chlorophyll electronic analyzer (CLOROFILOG CFL 1030, FALKER, serial 0520, Brazil). For that, 18 leaves in each treatment per block were measured in a total of 54 leaves per treatment in all experiment.

143 **Normalized difference vegetative index (NDVI)**

144 As an important indicator of the status of chlorophylls, photosynthetic capacity and energy
145 absorption by plant canopy, NDVI was measured using analyzer (PlantPen NDVI 300, Photon
146 System Instruments, Brazil).

147

148 **Leaf Gas exchange data**

149 Photosynthetic parameters were measured using a portable photosynthesis system-IRGA (LI-
150 6400XT-LI-COR, Biosciences Inc., Lincoln, NE, USA). Data of net photosynthesis (P_n), stomatal
151 conductance (g_s), intercellular carbon dioxide, transpiration, leaf to air temperature ratio, leaf
152 saturation vapor, total conductance to carbon dioxide, intercellular to ambient carbon dioxide,
153 intrinsic water use efficiency (WUE), stomatal limitation value (SL), carboxylation efficiency of
154 Rubisco were accessed. Intrinsic water use efficiency, stomatal limitation value and
155 carboxylation efficiency (CarbE) were calculated according to equations 1, 2 and 3 respectively.

156
$$\left[WUE = \frac{P_n}{g_s} \right] \text{ (Eq. 1)} \quad \left[SL = 1 - \frac{C_i}{C_a} \right] \text{ (Eq. 2)} \quad \left[CarbE = \frac{P_n}{C_i} \right] \text{ (Eq. 3)}$$

157 Where C_i and C_a means the intercellular and ambient carbon dioxide respectively, and g_s the
158 stomatal conductance.

159

160 Using “plantecophys”-an R package for analysing and modeling leaf gas exchange data [22],
161 photosynthesis and stomatal conductance models were fitted in order to better understand
162 and quantify inter-specific differences in photosynthesis and model the rapid response to
163 changes in environmental drivers such as light, humidity and temperature.

164

165

166 ***Dry matter, root length, root volume and total leaf mass***

167 These parameters were evaluated at the final of the experiment, when plants reached the R5-
168 R6 phenological stage according to Fernández [23]. Dry matter of leaves was measured by
169 weighing the fresh and dry weight (Forced air oven, 72 hours, 60⁰C) of sample and represented
170 as proportion of dry and wet sample weight (%), total fresh leaf mass by weighing all leaves
171 collected per treatment and represented in grams, root length using a rule and root volume by
172 the water volume dislocated by the roots.

173

174 ***Antioxidant enzymatic system (catalase-CAT and ascorbate peroxidase-APX)***

175 Enzymes were extracted from 1g of leaf tissue using a mortar and pestle with 5ml extraction
176 buffer containing 0.2M K-phosphate buffer pH7, 20mM DTT, 0.100g PVP, 25µL triton, 50mM
177 PMSF and 5mM EDTA. The homogenate was centrifuged (3000 rpm/30 minutes) and the
178 supernatant fraction was used to assay catalase, proteins and ascorbate peroxidase [24].
179 Protein content was determined using Bradford reagent [25] with bovine serum albumin as a
180 standard. Catalase activity was assayed in a reaction mixture of 2mL of 50mM pH7 phosphate
181 buffer containing hydrogen peroxide (25µL) and 20µL of enzyme extract. The decomposition of
182 hydrogen peroxide was followed at 240nm ($\epsilon=36 \text{ mM}^{-1} \text{ cm}^{-1}$). Total APX activity was measured
183 by monitoring the decline in absorbance at 290 nm, as ascorbate ($\epsilon= 2.8 \text{ mM}^{-1}\text{cm}^{-1}$) was
184 oxidized, for 2 min using the method of Nakano and Asada [26]. The assay medium consisted of
185 600 µL of 50 mM K-PO₄ buffer (pH 7.0) with ascorbate, 600 µL of buffer with hydrogen peroxide
186 and 300 µL of sample extract. APX activity was expressed in mM ascorbate.min⁻¹mg⁻¹ of
187 proteins.

188

189 ***Non-enzymatic antioxidant system (carotenoids, total phenolics and flavonoids) and UV-***
190 ***visible scanning of phenolic extracts***

191 Carotenoids were determined by the method described by Lichtenthaler & Buschman [27] using
192 cold acetone as a solvent and calculated by Lambert-Beer equations (4-6) respectively.

193
$$\left[C_{\text{carot}} (\mu\text{g/ml}) = (1000A_{470} - 1.90C_a - 63.14C_b) / 214 \right] (4)$$

194 The total phenolic contents of were determined by Folin-Ciocalteu reagent (FCR) method. For
195 a 2.0 mL total volume, 200 μ L of extract were first mixed with 100 μ L of FCR reagent after
196 adding 1.40 mL distilled water and the contents were kept at room temperature for 10 minutes.
197 Later, 300 μ L of Na₂CO₃ aqueous solution (20%) were added and incubated for 1 hour. The
198 absorbance was measured at 765 nm using a spectrophotometer. Total phenolics content was
199 expressed as μ g of gallic acid equivalents/g of dry extract (μ g GAE/g) using a standard curve (0-
200 1000 μ g/mL) of gallic acid [28].

201 UV-visible (UV-vis) scanning (200-800nm) using spectrophotometer of phenolic extracts was
202 also performed. Spectra were normalized, baseline corrected, subjected to feature selection at
203 the region of interest and non-supervised multivariate analysis (hierarchical cluster analysis-
204 HCA and principal component analysis-PCA).

205

206 Total flavonoid content of plant extract was determined using aluminum chloride colorimetric
207 method (Woisky & Salatino [29] and revised by Chang [30]) and standard solutions (0-1000
208 μ g/mL of quercetin in 80% methanol). For that, 1 mL extract solution was mixed with 0.5 mL
209 95% ethanol (v/v), 0.1 mL 1M potassium acetate, 0.1 mL aluminum chloride solution (10%
210 AlCl₃), and 0.8 mL distilled water to a total volume of 2.5 mL. The mixture was well mixed and
211 incubated at room temperature for 30 minutes versus reagent blank containing water instead

212 of sample. Quercetin was used as the standard for the quantification of total flavonoid. Results
213 were expressed as milligrams of quercetin equivalent per gram of dry weight (mg QE/g).

214

215 ***Data mining and statistics***

216 Data were summarized and subjected to normality (Shapiro-Wilk) and homogeneity (Levene)
217 tests and analysis of variance (Two-way ANOVA). Tukey HSD ($p < 0.05$) test was used where
218 differences were found. Multivariate statistical techniques were also used in other to find
219 important variables related to potassium in common beans. All analyses were performed in R
220 software [31] using scripts produced by our group. A report in html format is provided as
221 supplementary data for data analysis reproducibility. Leaf Gas exchange models were fitted
222 using Plantecophys package [22]. Equations were made in Mathtype software.

223

224 **Results and Discussion**

225 ***Growth parameters (total fresh leaf mass, dry matter, root length and root volume)***

226 Results of growth parameters are summarized in the figure 1(A-D). Two-way ANOVA of the
227 total fresh leaf mass showed differences between potassium levels in Dama cultivar. 10mM of
228 potassium nutrition provided major total fresh leaf mass ($p < 0.05$) and 1mM the minor value of
229 leaf mass. All other levels (0, 2, 20 mM) were statistically similar (Fig. 1A). Significant statistical
230 differences were found between potassium at 10Mm and other levels ($p < 0.05$). Increases in
231 potassium promoted the total leaf mass of “Dama” cultivar until level 10. For “Uirapuru”
232 cultivar, non-significant differences were found (Fig. 1A). Previous results by Yaghub [32] also
233 reported improvement of leaf mass by K application. Contrarily, K deficiency was found to
234 decrease the leaf number, leaf area and reproductive dry weight [10]. These changes explain

our results. Potassium deficiency affects the carbon-nitrogen balance. Improved plant survival and reduction of shoot elongation were also reported to be affected by K addition [11].

The dry matter of leaves (Fig 1B) differed between cultivars and potassium levels and was affected adversely by potassium. Independently of cultivar, the dry matter decreased gradually (1.5 fold and 1.4 fold) with increases of potassium levels in 'Dama' and 'Uirapuru' respectively from 1 to 20mM of potassium (Fig 1B). The 'Uirapuru' cultivar presented higher dry matter (16.7% fold) than 'Dama' ($P < 0.05$). Tukey HSD test ($p < 0.05$) showed significant differences between levels 1 and 2 with 10 and 20 in 'Dama' and between (2 and 10) with (1 and 20) in 'Uirapuru' (see supplementary data for details). Similar results were reported by Taibi [33] testing the effect of salt stress (NaCl) in *Phaseolus vulgaris*. In their research shoot and root biomass decreased with increases of salinity. K induces phenotypic dry matter allocation plasticity and was reported to be responsible of carbon allocation to osmotic adjustment [34]. Root length and volume showed a direct relationship between potassium levels. When K concentration increased until 10mM, the root length and volume (Figs. 1C and 1D) increased in cultivar dependent manner. In 'Dama' cultivar increases were observed until level 10 of potassium and in 'Uirapuru' until 2mM (Figs 1C-D). Besides, root length did not differ statistically in 'Dama' cultivar ($p < 0.05$). Similar trends were also observed for root volume (see supplementary data). In *Sulla carnosa*, a forage legume the growth of vegetative organs was decreased by 50% by K deficiency being the stems more affected than roots and leaves [35].

When Pearson correlation of all variables studied ($p < 0.05$) was done (Table 1), the total leaf mass (TLM), root volume (RV) and root length (RL) were found to be positively correlated with chlorophyll contents (Table 1) and these last two also correlated with catalase activity (Table 1). The dry matter content of leaves was negatively correlated with carotenoid content and TLM.

259 ***Photosynthetic pigments, enzymatic and non-enzymatic antioxidants***

260 ***Chlorophyll A and B***

261 Chlorophyll A and B increased with potassium application (Fig 2A-B) in all cultivars. The
262 accumulation of chlorophylls was higher in 'Dama' cultivar than 'Uirapuru' ($p < 0.05$). Increases
263 in chlorophyll contents may be attributed to the role of K in plant metabolism. Essentiality of K
264 in plant life cycle is evidenced by its role in activating enzymes which are involved in various
265 physiological processes such as energy metabolism, starch synthesis, nitrate reduction,
266 photosynthesis and sugar degradation. As a component of plant cytoplasmatic solution, K
267 reduces loss of water from leaf surfaces by regulating stomatal closure and increases water
268 uptake efficiency of roots from soil mainly due to the role of K in regulating cellular osmotic
269 potentials [36] which may explain our observed results. Cultivar 'Dama' was most efficient in
270 activating chlorophyll pigments.

271
272 ***Catalase activity (CAT)***

273 CAT in both genotypes increased until 2mM of potassium and then decreased gradually (Fig
274 2C). At higher levels of potassium, CAT was less important as enzymatic antioxidant system. The
275 activation of CAT was markedly in 'Dama' cultivar than 'Uirapuru'. 'Dama' exhibited plants with
276 better performance may be due to the activation of many antioxidant mechanisms. Contrarily,
277 the level of ascorbate peroxidase (APX) was higher in 'Uirapuru', which decreased with
278 potassium levels (Fig 2D). Trace amounts of APX were found in 'Dama'. Results indicated that
279 these 2 enzymatic systems may be of lesser importance in all genotypes at higher rates of
280 potassium.

281

282

283 ***Carotenoids***

284 Carotenoids (Fig 2E) increased with potassium levels. The rate was higher in 'Dama' than
285 'Uirapuru'. Carotenoids, being antioxidants, have the potential to detoxify the plants from the
286 effects of reactive oxygen species. Carotenoids are known to function as collectors of light
287 energy for photosynthesis and as quenchers of triplet chlorophyll and O₂. Moreover, they
288 dissipate excess energy via the xanthophyll cycle and can act as powerful chloroplast
289 membrane stabilizers [33, 37, 38]. Increases in carotenoid contents indicate that those
290 compounds play an important role under increases of potassium levels. As reported by
291 Cazzonelli & Pogson [39], carotenoids play essential roles in development, photosynthesis, root
292 mycorrhizal interactions and the production of phytohormones, such as abscisic acid and
293 strigolactones.

294

295 ***Flavonoids***

296 For flavonoid contents (Fig 2F), increases were observed only for 'Dama' cultivar. Flavonoids
297 protect the plant against ultraviolet radiations, have antimicrobial properties and act as a
298 deterrent for herbivores by limiting assimilation of dietary proteins and inhibiting digestive
299 enzymes [40]. Furthermore, flavonoids act as scavengers of free radicals such as reactive
300 oxygen species (ROS), and also prevent their formation by chelating metals [41-44, 40].
301 Increases in flavonoids observed in our research may be related to antioxidant functions of
302 those compounds mainly in 'Dama' cultivar which exhibited plants with better performance.

303

304 ***Total phenolics***

305 Regarding the phenolic content, decreases were observed until level 2 of potassium and then
306 increased in Dama cultivar. Contrarily, for 'Uirapuru', an increase until 2mM and then

307 decreased gradually (Fig 2G). Phenolics were observed to play a role only for 'Dama' Cultivar at
308 higher levels of potassium. This group of compounds is claimed to have the ability of free-
309 radical scavenging and are essential to plant physiology and resistance to biotic and abiotic
310 stresses. Besides removing free radicals, these compounds are capable of chelating metal,
311 activating antioxidant enzymes, and inhibiting oxidases [41].

312

313 ***Normalized difference vegetation index (NDVI)***

314 NDVI results are summarized in the figure 2H. As it can be observed, NDVI index increased
315 gradually with potassium levels until 10mM. Live green vegetation was higher in 'Dama' cultivar
316 than 'Uirapuru' but was statistically non significant ($p < 0.05$, Tukey HSD test). The results were
317 in accordance with the total biomass observed by that Cultivar. NDVI was higher in control
318 plants than those at 20mM of potassium and was positively correlated with carotenoids,
319 flavonoids, net photosynthesis, stomatal conductance, leaf saturation vapour and the
320 carboxylation efficiency and negatively correlated with chlorophyll contents (See Table 1).
321 Similar results were also reported by Penuelas [44] studying physiological changes in nitrogen
322 and water limited sunflower leaves. Positive correlations with chlorophyll and secondary
323 metabolites were also reported previously in aquatic vegetation [45-46]. NDVI was also
324 positively correlated with leaf area index [47]. NDVI is a broadband index (i.e., normalized
325 differences between the reflectance in the near infrared and the red regions of the light
326 spectrum) that is related to green biomass and has been used to indirectly estimate
327 photosynthetic capacity and net primary productivity [48] and assess whether the target being
328 observed contains live green vegetation or not. NDVI correlates well with canopy features such
329 as biomass, leaf area index (LAI), absorbed photosynthetically active radiation, and canopy

330 photosynthetic capacity, but fails to capture dynamic physiological processes, which may occur
331 on fine temporal and spectral scales [49,48]

332

333 ***UV-visible scanning of phenolic extracts***

334 All sample spectra showed higher absorbance in the similar wavelength regions (300-500nm -
335 region of phenolics and carotenoids and 640-700nm (see supplementary Figure 1). Those
336 regions were selected for further multivariate analysis. HCA analysis showed four different
337 groups. 'Dama' cultivar samples with 1 and 10mM of potassium separated alone in distinct
338 groups. The third group was composed mainly by 'Uirapuru' cultivar samples with 1, 2, 10,
339 control and 'Dama' with 20mM. The fourth group was composed by 'Dama' cultivar samples
340 (control, 2mM) and 'Uirapuru' with 20mM of potassium. The cophenetic correlation of HCA was
341 88% according to Euclidean distance (Figure 3A).

342 Principal component analysis showed that samples of cultivar 'Dama' with potassium levels of
343 1mM and 10mM separated alone due to their composition in chlorophyll contents as it can be
344 observed by the loadings or Eigen values of PCA (Figure 3B). Other samples were similar due
345 their composition in phenolics and carotenoids. The total variance explained by PCA was 88.3%,
346 being 48.6 and 33.7% for component 1 (PC1) and 2 (PC2) respectively.

347

348

349 ***Leaf Gas-exchange data***

350 Results of leaf gas exchange are summarized in the Table 2. The net photosynthesis of all
351 cultivars increased gradually with potassium levels from 1 to 10mM. The rate of photosynthesis
352 was higher in 'Uirapuru' cultivar except for control plants. A slight increase in stomatal
353 conductance was observed for 'Dama' cultivar. Higher rate of stomatal conductance at 10mM

of potassium was observed for 'Uirapuru'. Intercellular carbon dioxide decreased in all cultivars but the rate was higher for 'Uirapuru' at level 10 of potassium. The rate of transpiration in all cultivars increased with potassium until level of 10mM. The rate of transpiration was higher in 'Uirapuru'. The leaf to air temperature and leaf saturation vapor showed slight variation in plants treated with different potassium levels, except at level 10 where a maxima was observed in 'Uirapuru' cultivar. The total conductance to carbon dioxide showed similar trend as in stomatal conductance previously mentioned in the figure 4B. The rate of intercellular to ambient carbon dioxide decreased in all cultivars, but the decreasing rate was higher at 10mM of potassium for 'Uirapuru' cultivar. Cultivar 'Dama' showed water use efficiency and superior stomatal limitation value except at 10mM of potassium. Higher rubisco carboxylation was also observed at 10mM of potassium in 'Uirapuru'.

Photosynthetic assimilation of carbon is a defining feature of plant kingdom. The fixation of large amount of carbon dioxide supports the synthesis of carbohydrates, which make up the bulk of plant biomass. Measurements of carbon assimilation rates are therefore crucial due to their impact on the plant metabolism, growth and reproductive success [50]. As it can be observed in our results, potassium nutrition in the range of (1-10mM) promoted higher carbon assimilation. The decrease of intercellular carbon dioxide may be attributed to the higher transpiration rate and assimilation activity observed. Cultivar 'Dama' was most efficient in water use and showed lower stomatal limitation value. Higher carbon assimilation rates in 'Uirapuru' cultivar may be attributed to the elevated stomatal limitation value. The carboxylation of rubisco was more pronounced at 10mM of potassium in all cultivars. It is important to note that the presence of finite stomatal resistance to carbon dioxide cause carbon dioxide at sites of carboxylation to be less than that in the atmosphere, thereby

378 reducing the rate of carbon dioxide assimilation somewhat below its potential. Nevertheless,
379 stomata usually impose the largest resistance to diffusion [51]. Lower stomatal values in 'Dama'
380 cultivar may have contributed to lower carbon dioxide assimilation rates observed in this
381 research. Previous report has indicated that K deficiency promotes inhibition of enzymatic-
382 photochemical processes [52]. Results indicate lower chlorophyll content and altered Rubisco
383 activity as probable causes of photosynthetic impairment. Potassium deficiency was found to
384 diminish photoprotection mechanisms due to reduced photosynthetic and photorespiratory
385 capacity [52].

386

387 In the past, stomatal responses have generally been considered in relation to single
388 environmental variables in part because the interactions between factors have appeared
389 difficult to quantify in a simple way. A linear correlation between stomatal conductance (g_s)
390 and carbon dioxide assimilation rate has been reported when photon fluence was varied and
391 when the photosynthetic capacity of leaves was altered by growth conditions, provided CO_2 , air
392 humidity and leaf temperature were constant [53]. In this research using the measured g_s we
393 fitted the stomatal conductance models of the two cultivars (Fig. 4A-B). A linear ('Dama'
394 cultivar) and non-linear regression was found for 'Uirapuru' cultivar (Fig 4A-B). As we know,
395 fundamental to any examination of stomatal functioning is the understanding of how rate of
396 assimilation of carbon dioxide responds to potassium levels. Furthermore, stomatal movements
397 provide the leaf with the opportunity to change both the partial pressure of carbon dioxide at
398 sites of carboxylation and the rate of transpiration [51, 54-56]. For 'Dama' cultivar (Fig. 4B) at all
399 levels of potassium, the fitted models showed a linear relation, but such trend was not
400 observed for 'Uirapuru' (Fig 4A).

401

402 Data of all variables studied were also correlated (Pearson correlation, $p < 0.05$) aiming to find an
403 association between them. As it can be observed in the Table 1, a positive correlation of root
404 length with chlorophylls was found. Significant positive association was also observed in net
405 photosynthesis with stomatal conductance and carboxylation efficiency (Table 1). The stomatal
406 conductance was negatively correlated with the intercellular carbon dioxide and intrinsic water
407 use efficiency. The transpiration rate was positively correlated with leaf temperature, leaf
408 saturation vapor and stomatal conductance. Contrarily, a negative association of NDVI index
409 with chlorophyll contents was observed (see Table 1). A positive correlation between the
410 stomatal limitation value with the carboxylation efficiency of rubisco was also found.

411
412 When all dataset was subjected to principal component analysis (PCA) aiming to dimensionality
413 reduction and find important variables, similarities and dissimilarities between the samples (Fig.
414 5), an interesting result was found, as it can be observed in the score plot and loadings of PCA.
415 Control samples grouped together showing that there are similar and composed the first group
416 in PCA. Such an observation was also found for those samples where 1mM of potassium was
417 applied. Samples of 'Uirapuru' cultivar with 2 and 20 mM of potassium grouped together. A
418 clear separation was observed from 'Uirapuru' at 10mM of potassium and the control samples.
419 Samples of 'Dama' cultivar at 2, 10 and 20 grouped also together due to their catalase activity
420 and intrinsic water efficiency presented. Intercellular carbon dioxide and the ratio of
421 intercellular to ambient carbon dioxide were the parameters that most contributed to sample
422 clustering of control plants (see Fig 5). Contrary, 'Uirapuru' samples at 10mM stayed alone due
423 to phenolic contents, high level of NDVI index, stomatal conductance, net photosynthesis,
424 transpiration and stomatal limitation value. The total variance explained by the model was
425 64.3%, being 36.6 and 27.7% for component 1 (PC1) and 2 (PC2) respectively. Most samples

grouped in (PC1-/PC2-) axis. The control samples grouped in the (PC1-/PC2+) axis and 'Uirapuru' at 10mM in (PC1+/PC2+) axis.

The loading values indicated that sample clustering in PC1 were highly influenced by the net photosynthesis, stomatal conductance, intercellular carbon dioxide, transpiration, intercellular to air temperature, carboxylation efficiency and stomatal limitation value. Those clustered in PC2 were most influenced firstly by the chlorophyll contents, followed by catalase activity, phenolic contents, root length, root volume, transpiration, water use efficiency and NDVI index.

Conclusions

The results of this study prompt us to conclude that there are considerable photosynthetic and biochemical differences between the cultivars studied in their response to potassium nutrition. Potassium range from 1 to 10mM promoted the plant biomass, root length and volume and chlorophyll contents. Increases in carotenoids, flavonoids and catalase activity may be correlated to their antioxidant activities and as scavengers of reactive oxygen species that can be produced during the high photosynthetic rate and transpiration. Cultivar 'Dama' was most tolerant to potassium levels and presented a linear relationship of stomatal conductance. Potassium levels also increased net photosynthesis, NDVI indices, carboxylation efficiency and transpiration, but the variation was in a cultivar-dependent manner. Intercellular carbon dioxide decreased with increases of potassium levels. Multivariate tools revealed that most samples of 'Dama' cultivar were most influenced by CAT and the intrinsic water use efficiency presented than 'Uirapuru'.

450 ***Supplementary data***

451 A report in html format is provided for data reproducibility. Detailed statistical analysis not
452 shown in the manuscript can be found here.

453

454 ***Acknowledgements***

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456 thanks CAPES for supporting the postdoctoral fellowship under the PNPd program.

457

458 ***Author's contribution***

459 The first author designed the experiment, evaluated, performed the data mining in R software
460 and drafted the paper together with the last author. All other authors contributed equally in
461 data collection, analysis and discussion.

462

463 ***Conflict of interest***

464 None declared

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677 **Figure captions**

678 Table 1: Pearson correlations of all variables. Chla, Chlb and Chlt mean chlorophyll a, b and total
679 respectively. CAT-catalase activity; APX-ascorbate peroxidase activity; CAR- total carotenoids;
680 FLA-total flavonoids; PHE-total phenolic compounds; TLM-total leaf mass; DM- dry matter; RL-
681 root length; RV- root volume; TRA-transpiration rate; SVTleaf-leaf saturation vapor; Cond-
682 stomatal conductance; CndTotal -total stomatal conductance; WUE-intrinsic water use
683 efficiency; Ls-stomatal limitation value; Pn-net photosynthesis; CarbE- carboxylation efficiency
684 of Rubisco; Ci and Ca-intercellular and ambient carbon dioxide respectively; Ti and Ta-
685 intercellular and ambient temperature respectively and NDVI-normalized difference vegetative
686 index.

687

688 Table 2: Gas-exchange measurements of the two common bean cultivars. Net photosynthesis,
689 stomatal conductance, intercellular carbon dioxide, Transpiration rate, Leaf to air temperature
690 ratio, Leaf saturation vapor, Total conductance to carbon dioxide, Intercellular to ambient
691 carbon dioxide, Intrinsic water use efficiency, Stomatal limitation value and Carboxylation
692 efficiency of rubisco.

693

694 Figure 1: Total leaf mass (A), dry matter (B), root length (C) and root volume (D) of the two
695 cultivars subjected to potassium levels. Tukey HSD tests are presented in the supplementary
696 data. (E)-Detailed color images of the common bean cultivars used in the experiment.

697

698 Figure 2: Changes in chlorophyll A (A), chlorophyll B (B), catalase (C), ascorbate peroxidase (D),
699 total carotenoid contents (E), total flavonoids (F), total phenolic contents (G) and NDVI index

700 (H) with potassium levels for two common beans cultivars. Values are representative of mean
701 and standard error of the mean.

702

703 Figure 3. (A) Hierarchical cluster analysis of UV-Visible scanning spectra of all samples (B) Score
704 plot and loadings of principal component analysis (B).

705

706 Figure 4: Modeled (with the model of Medlyn et al., 2011) versus measured stomatal
707 conductance (gs) for the two cultivars studied under different potassium levels. (A) for
708 'Uirapuru' and (B) for 'Dama' cultivars.

709

710 Figure 5: Score and loading plot of Principal component analysis of all dataset showing sample
711 clustering and the main variables correlated. The total variance explained by the two first
712 components were 36.6% and 27.7% respectively.

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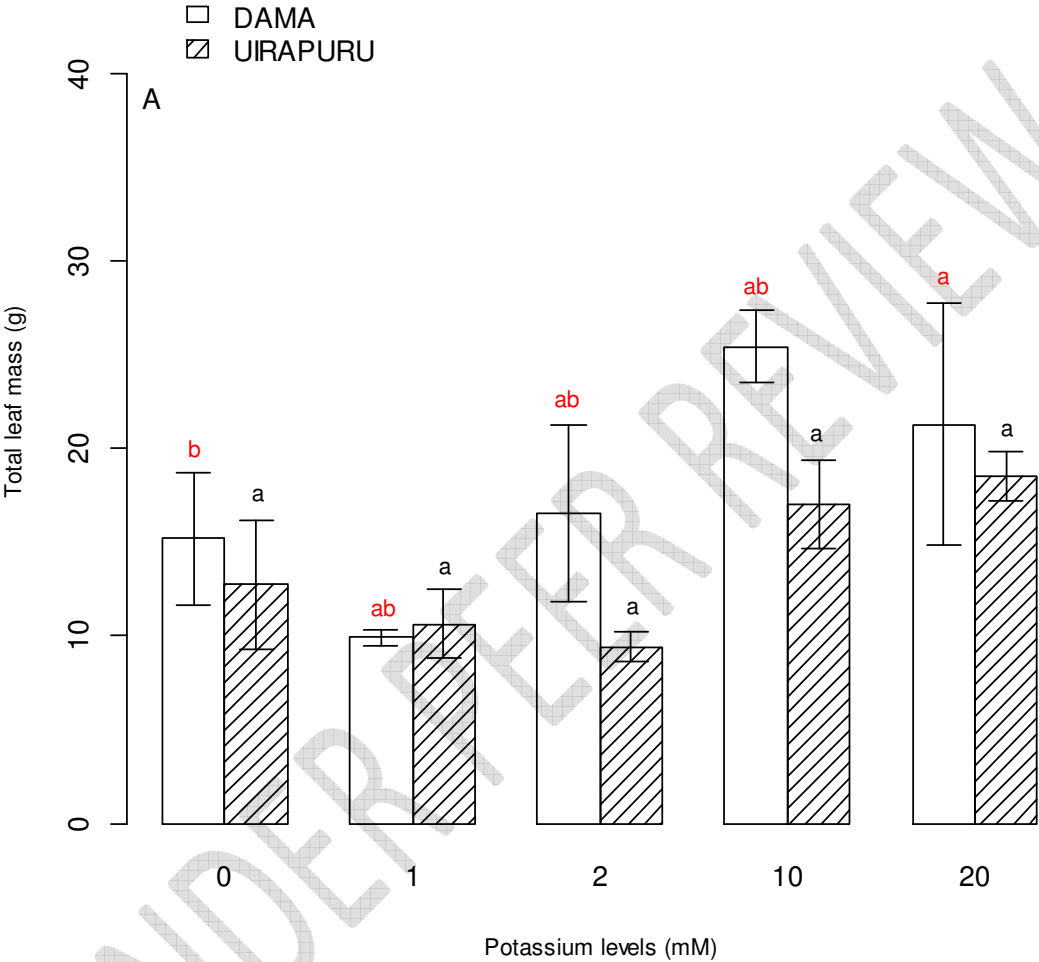
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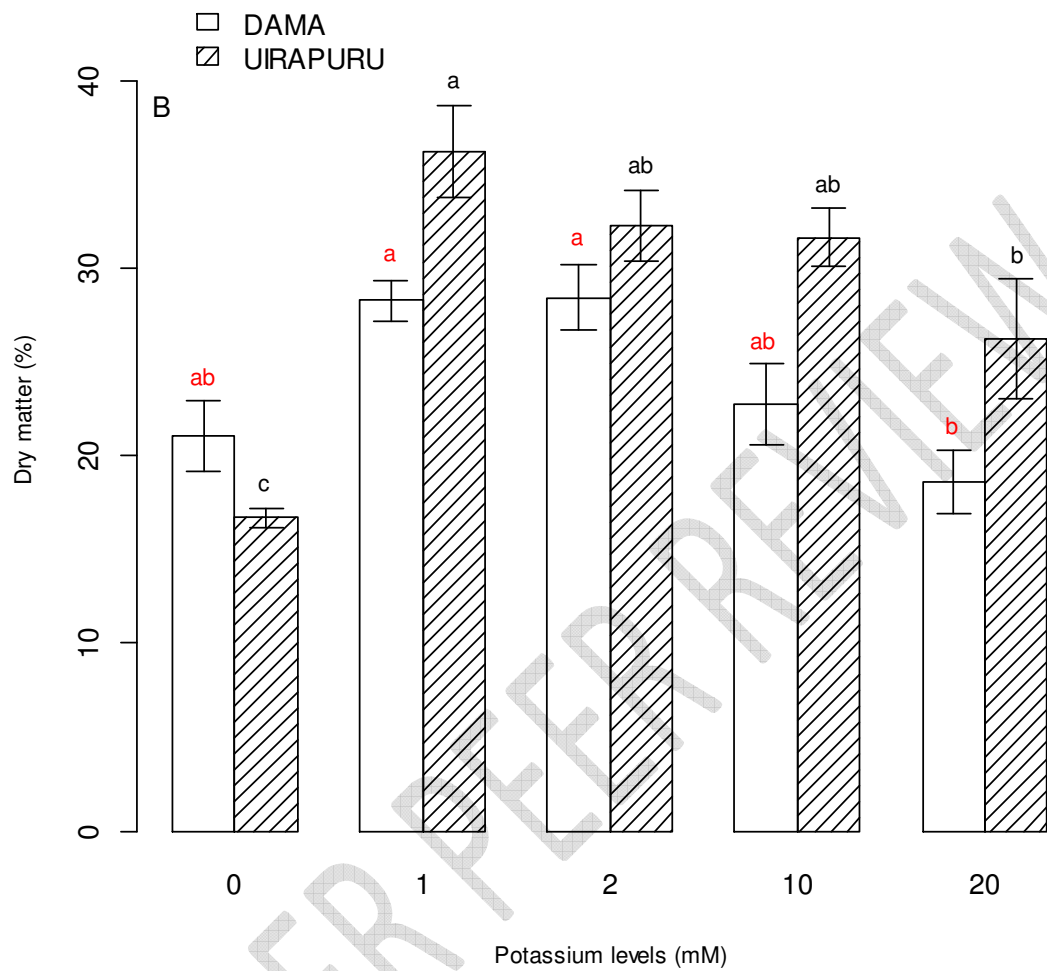
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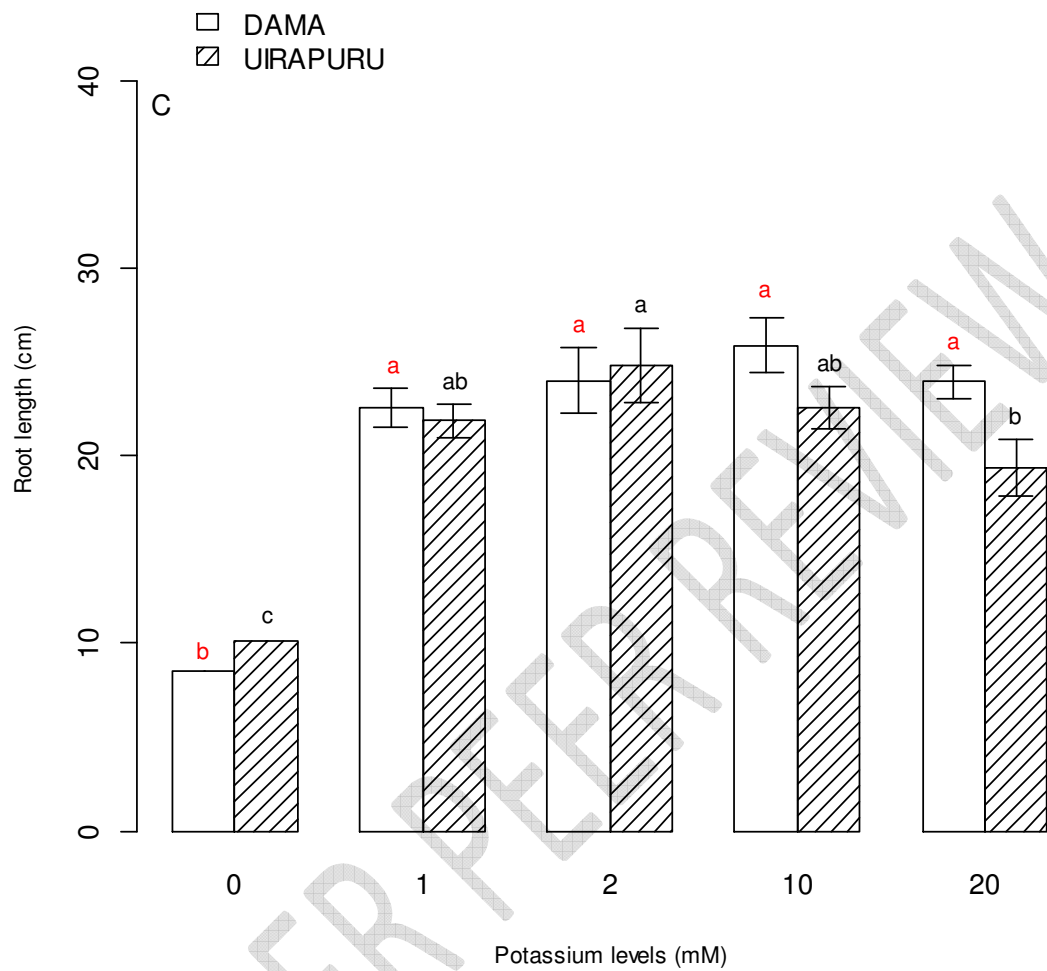
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725 Figure 1





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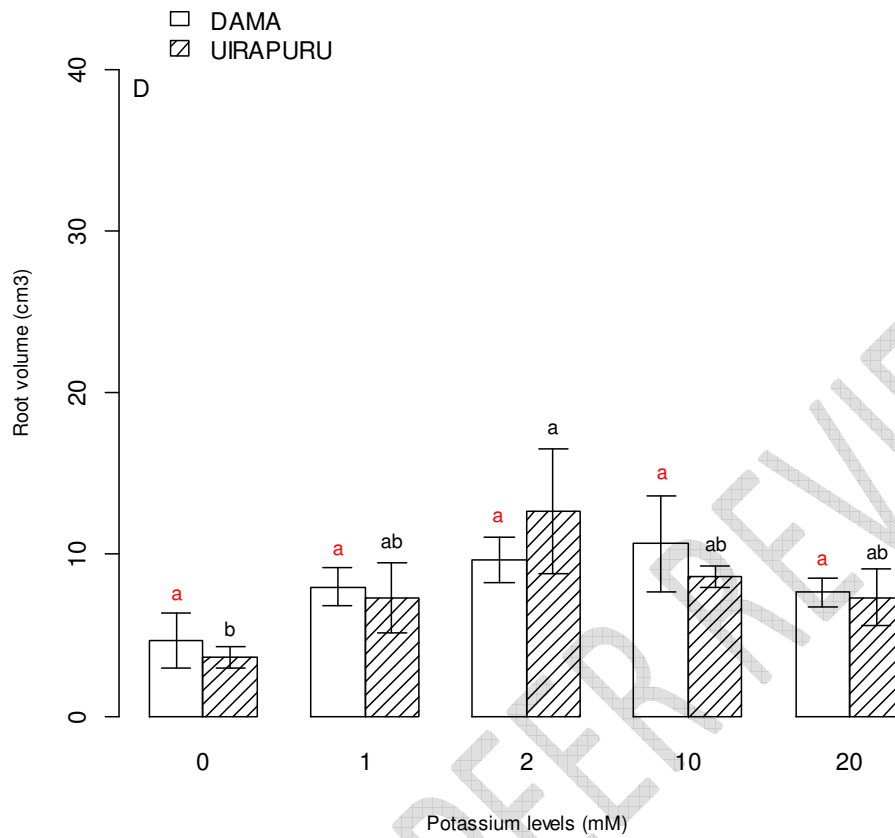
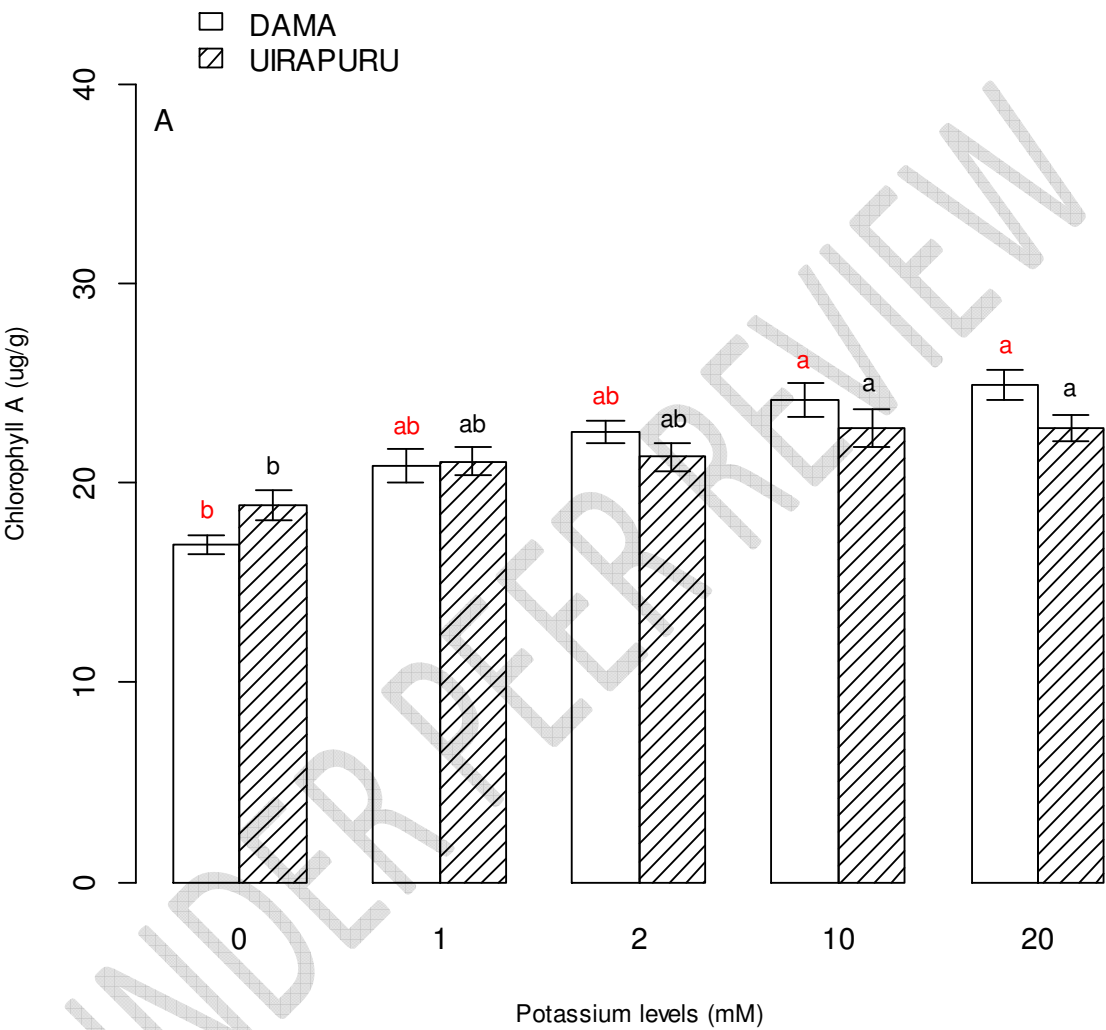
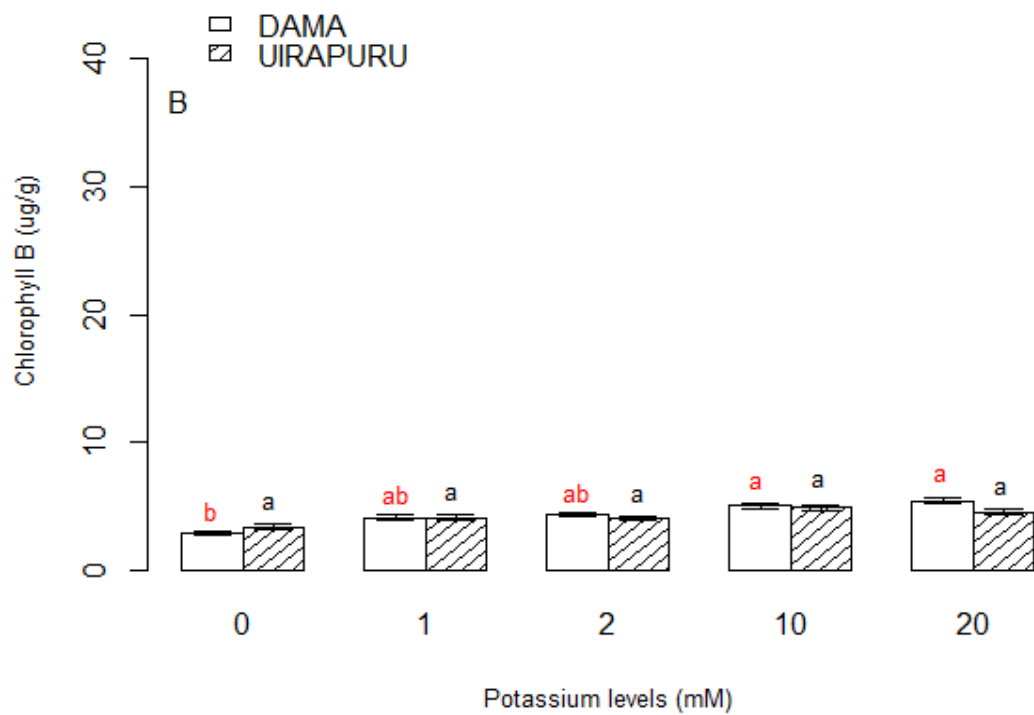


Figure 1E



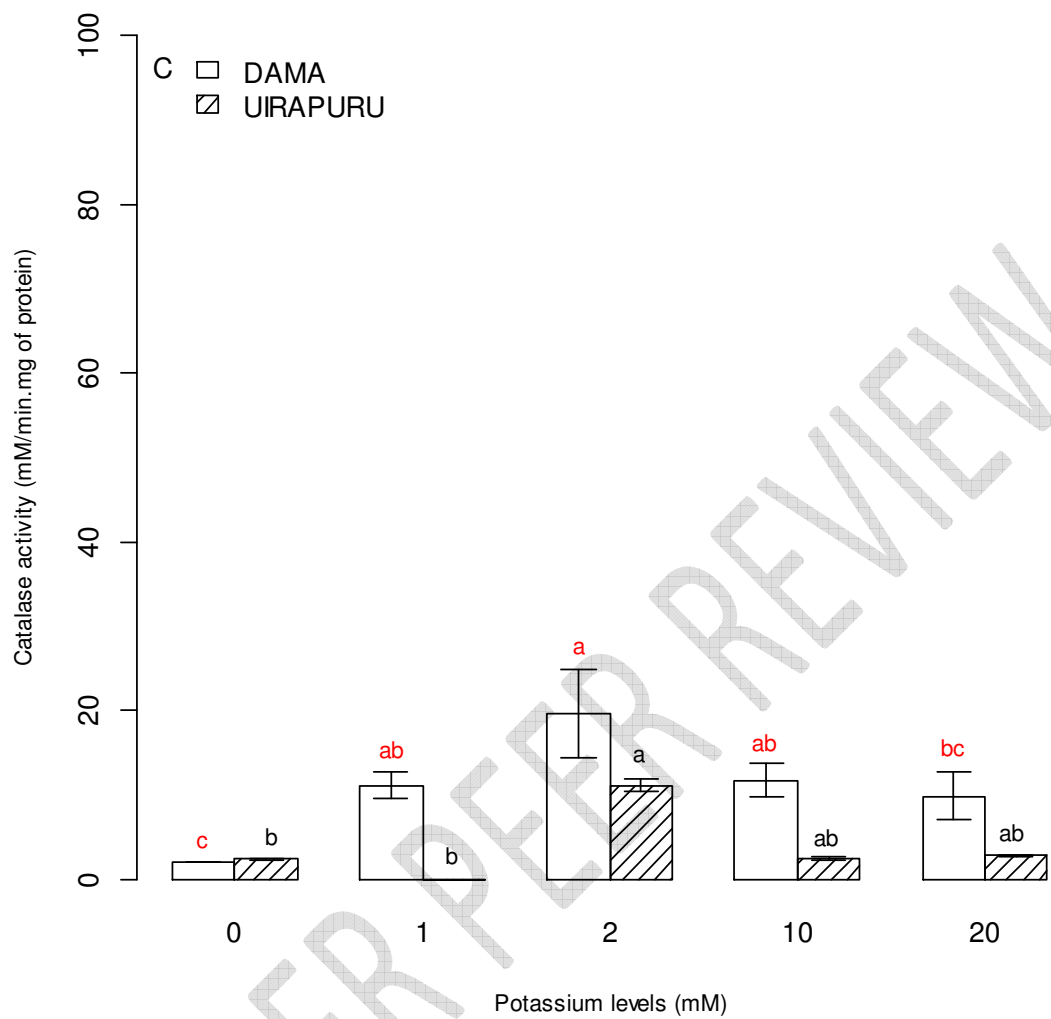
(A)- Cultivar TAA DAMA and (B)-IPR88 UIRAPURU.



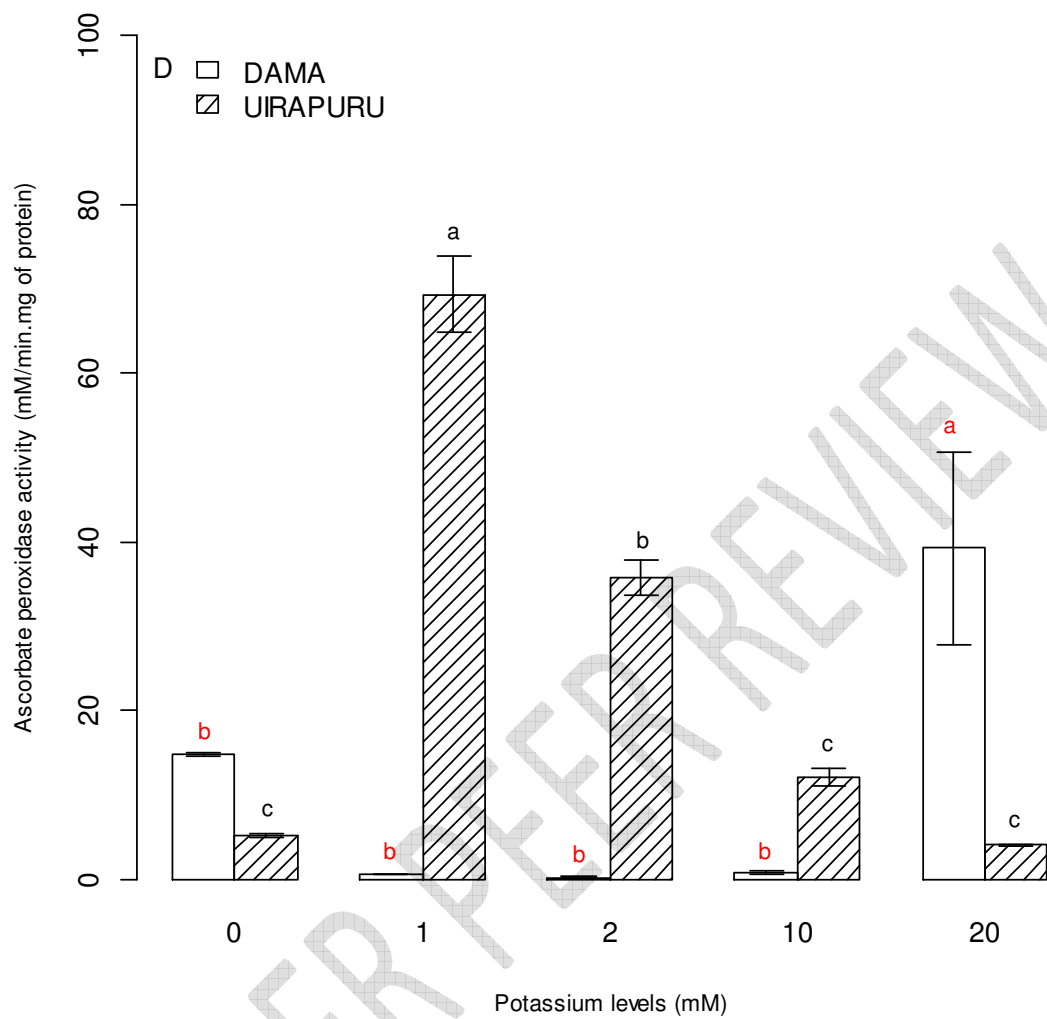


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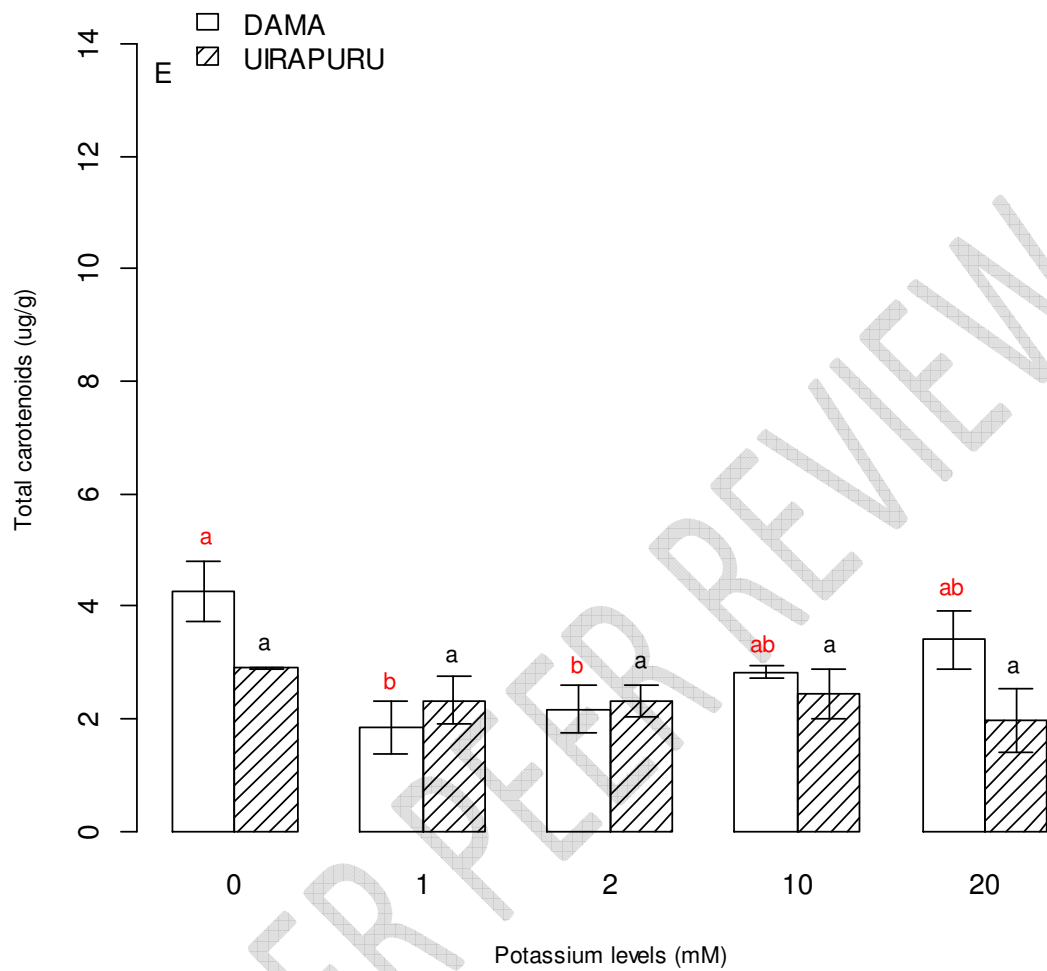
UNDER REVIEW



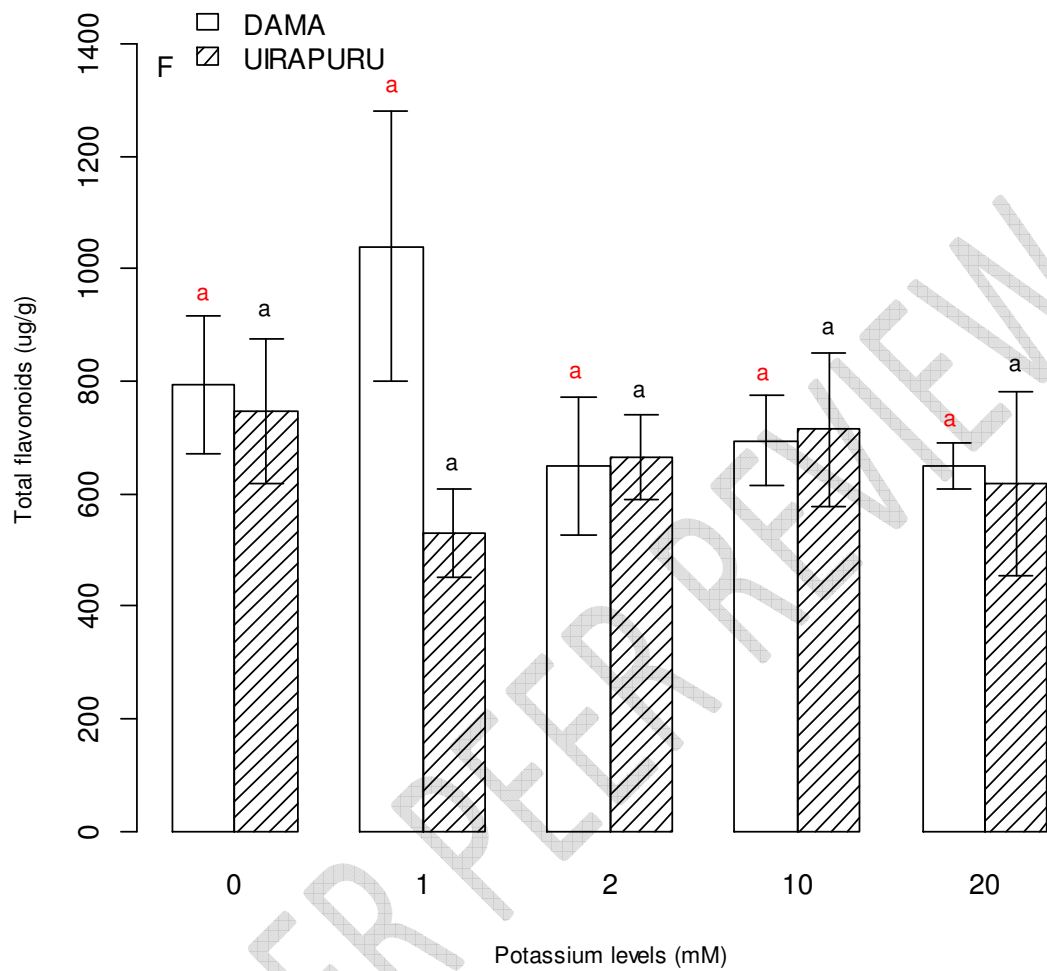
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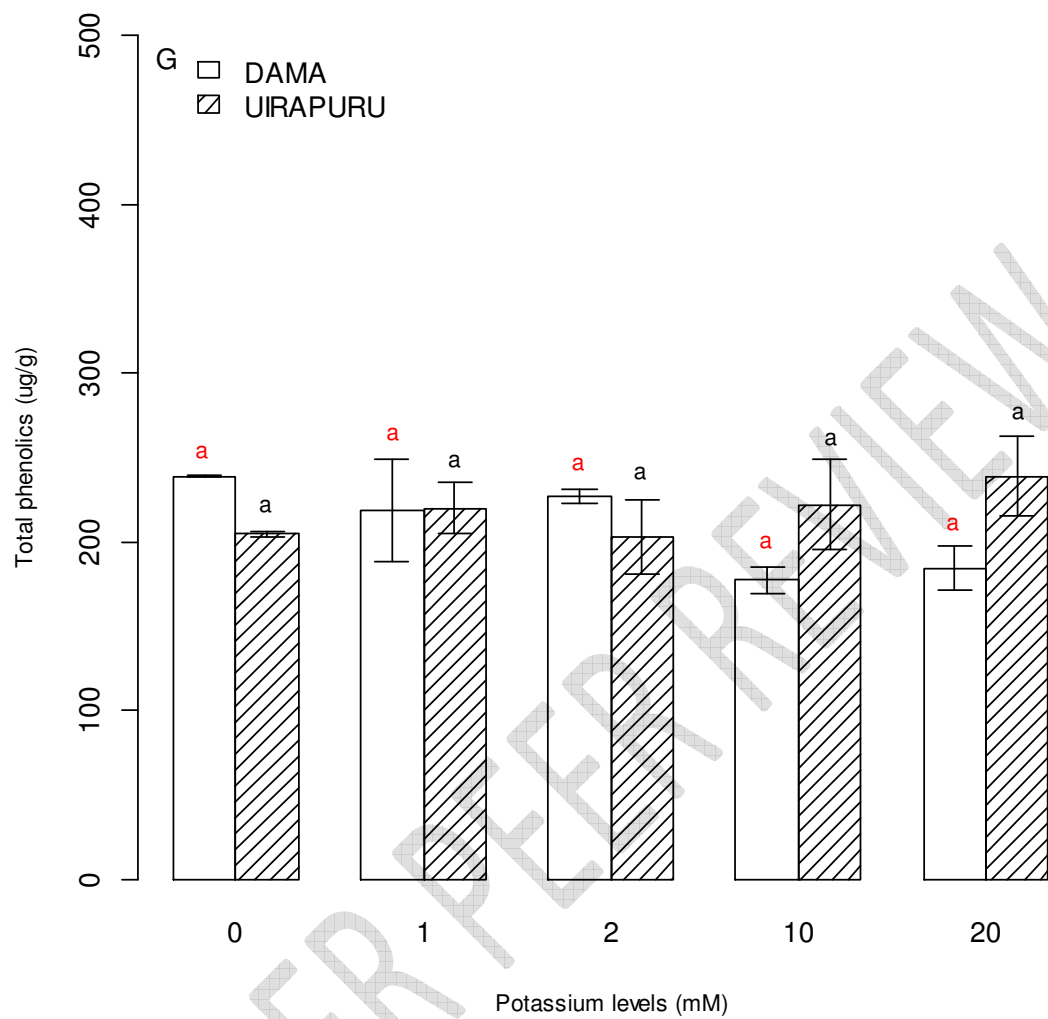
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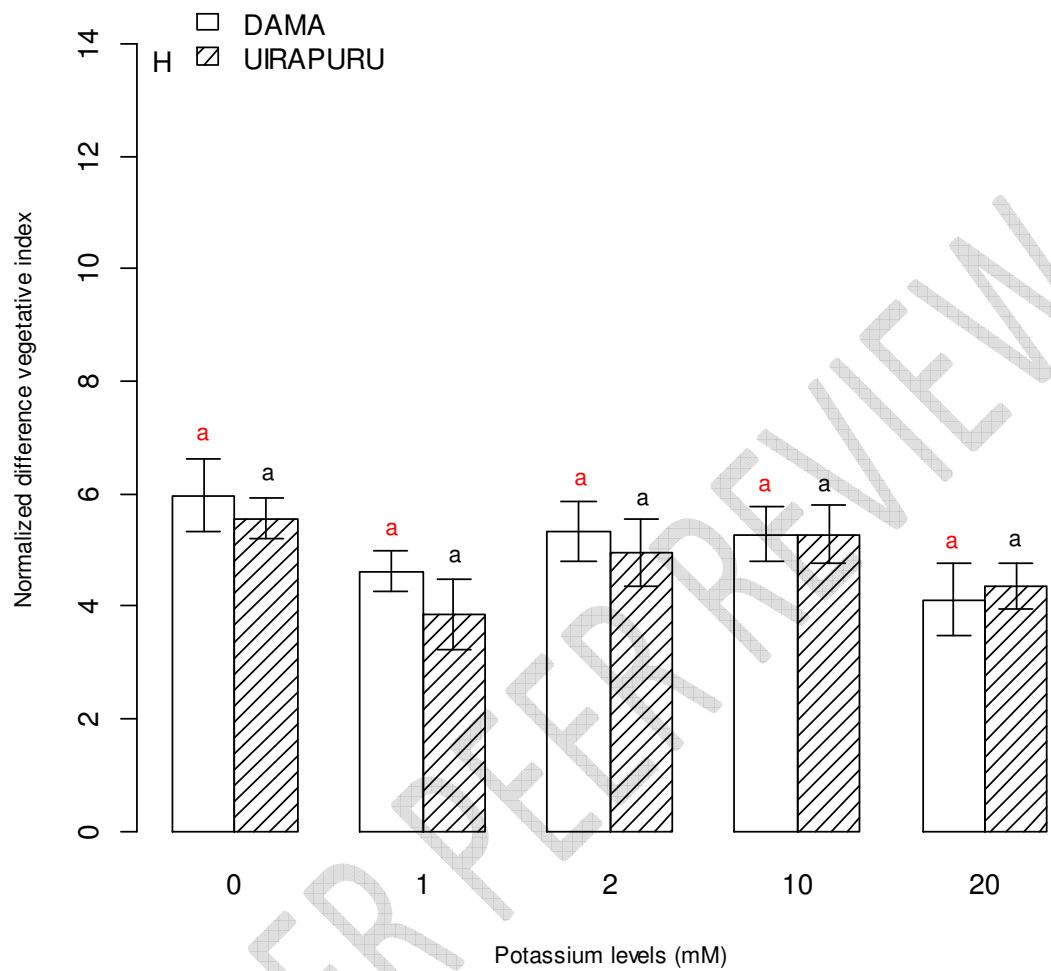
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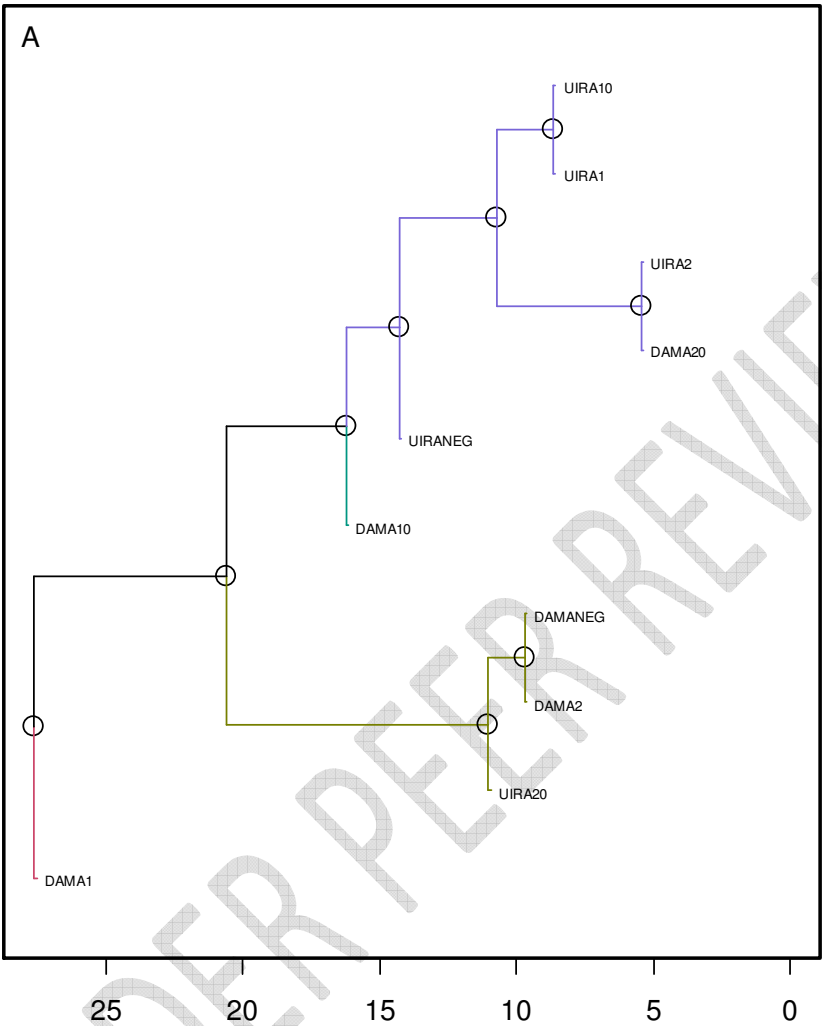
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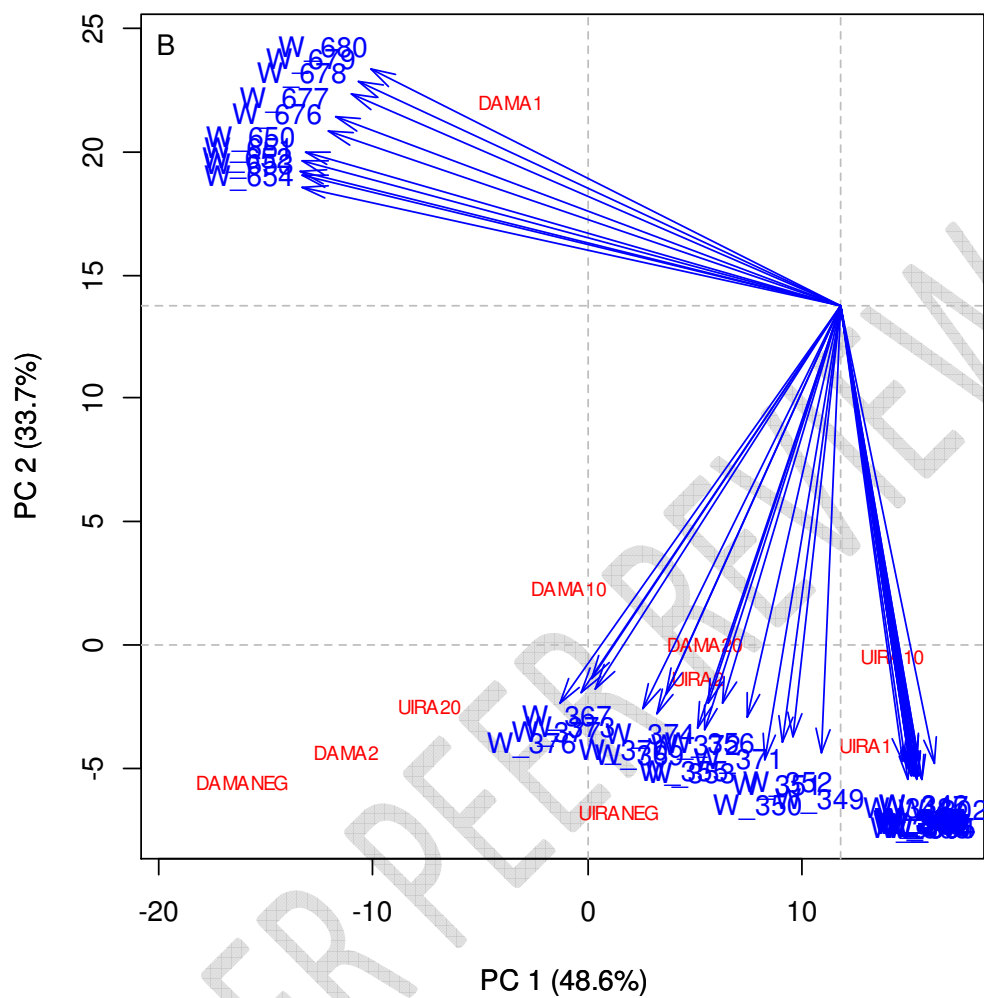
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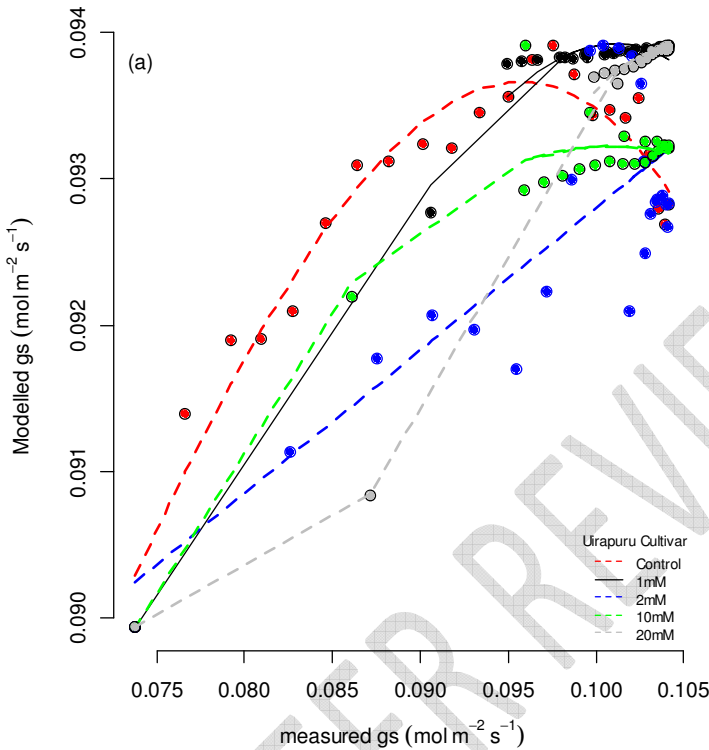
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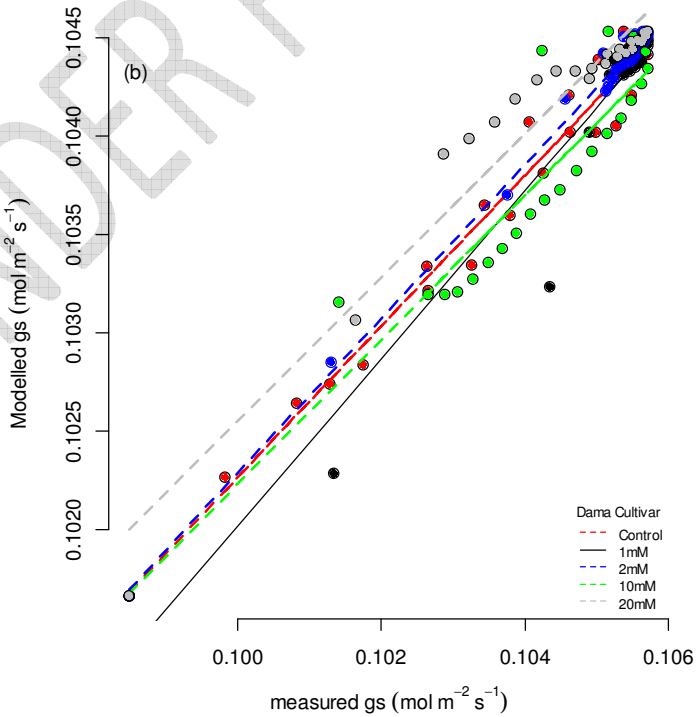




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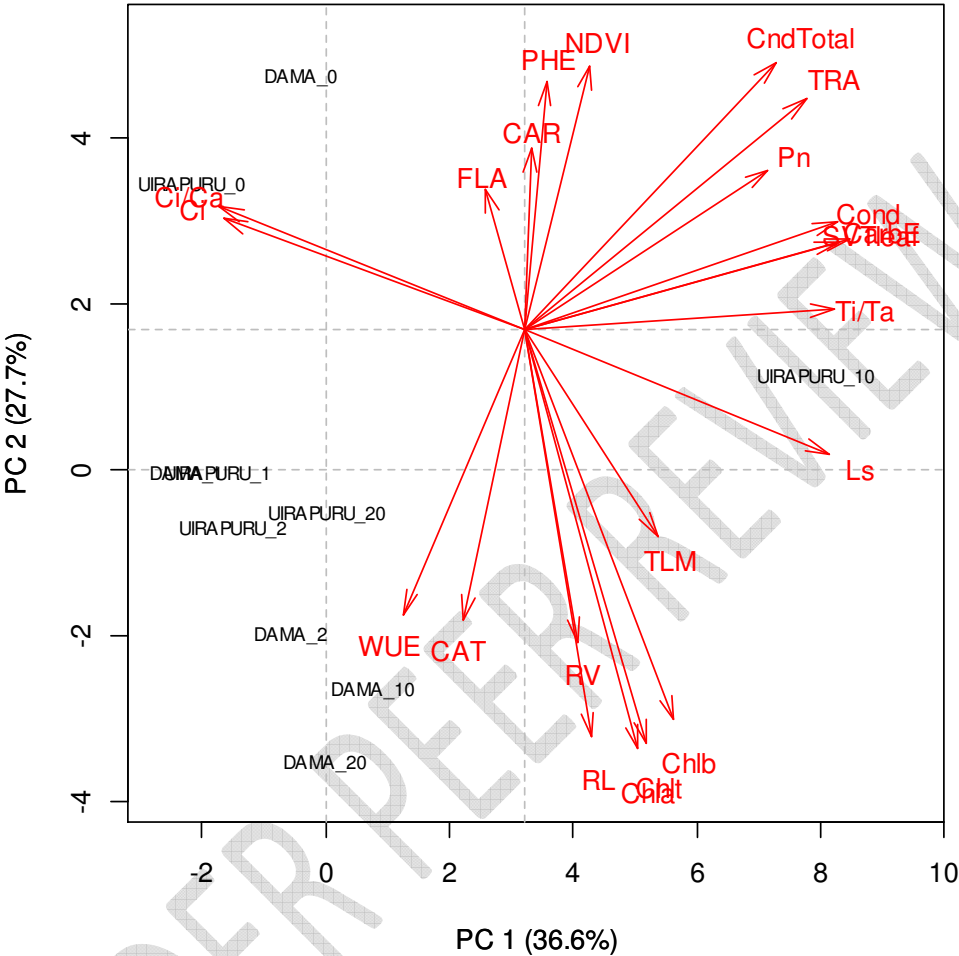


Table 1 **: Pearson correlations of all variables

	Chla	Chlb	Chlt	CAT	APX	CAR	FLA	PHE	TLM	DM	RL	RV	Pn	Cond	Ci	TRA	Ti/Ta	SVTleaf	CndTotal	Ci/Ca	WUE	Ls	CarbE
Chla																							
Chlb	0.98****																						
Chlt	1.00****	0.99****																					
CAT	0.43	0.32	0.41																				
APX	0.04	0.06	0.04	-0.34																			
CAR	-0.38	-0.31	-0.36	-0.26	0.10																		
FLA	-0.35	-0.30	-0.34	0.15	-0.54	-0.01																	
PHE	-0.52	-0.52	-0.52	-0.32	-0.13	-0.13	0.08																
TLM	0.61	0.63	0.61	0.19	-0.32	0.29	-0.26	-0.41															
DM	0.12	0.11	0.12	0.00	0.42	-0.62	-0.23	0.32	-0.46														
RL	0.85**	0.81**	0.84**	0.58	0.16	-0.58	-0.21	-0.43	0.23	0.52													
RV	0.59	0.53	0.58	0.62	0.07	-0.46	-0.18	-0.35	0.11	0.53	0.86**												
Pn	-0.09	0.02	-0.06	-0.46	-0.17	0.37	-0.13	0.31	0.41	0.02	-0.22	-0.08											
Cond	0.10	0.22	0.13	-0.33	-0.10	-0.04	0.00	0.17	0.08	0.27	0.05	0.04	0.64*										
Ci	-0.51	-0.59	-0.53	-0.10	0.21	-0.14	0.14	0.01	-0.62	-0.01	-0.30	-0.19	-0.62	-0.72*									
Tra	-0.17	-0.05	-0.14	-0.48	-0.18	0.20	0.03	0.22	0.08	0.07	-0.26	-0.15	0.76*	0.93****	-0.59								
Ti/Ta	0.27	0.38	0.30	-0.19	-0.07	-0.19	0.01	0.12	0.09	0.36	0.24	0.15	0.51	0.97****	-0.76*	0.81**							
SVTleaf	0.14	0.26	0.17	-0.28	-0.12	-0.05	0.03	0.15	0.11	0.24	0.08	0.03	0.60	0.99****	-0.76*	0.90****	0.98****						
CndTotal	-0.27	-0.16	-0.24	-0.58	-0.13	0.23	-0.04	0.24	0.04	0.08	-0.34	-0.18	0.80**	0.85**	-0.46	0.98****	0.70*	0.80**					
Ci/Ca	-0.54	-0.62	-0.56	-0.11	0.17	-0.09	0.16	0.02	-0.58	-0.07	-0.35	-0.24	-0.59	-0.74*	1.00****	-0.59	-0.79**	-0.78**	-0.46				
WUE	0.43	0.35	0.41	0.57	-0.04	0.19	-0.22	-0.26	0.55	-0.33	0.29	0.22	-0.23	-0.66*	-0.04	-0.69*	-0.58	-0.62	-0.71*	-0.01			
Ls	0.54	0.62	0.56	0.10	-0.18	0.06	-0.14	-0.01	0.58	0.08	0.35	0.24	0.59	0.74*	-1.00****	0.59	0.79**	0.78**	0.45	-1.00****	0.01		
CarbE	0.14	0.27	0.17	-0.34	-0.16	0.09	-0.04	0.22	0.30	0.19	0.03	0.04	0.83**	0.95****	-0.82**	0.91****	0.90****	0.94****	0.84**	-0.83**	-0.46	0.83**	
NDVI	-0.52	-0.51	-0.52	0.08	-0.61	0.45	0.33	0.14	0.09	-0.39	-0.52	-0.19	0.40	0.24	-0.15	0.49	0.08	0.22	0.50	-0.11	-0.22	0.09	0.26

Significance levels: $p < .0001$ '****'; $p < .001$ '***', $p < .01$ '**', $p < .05$ '*'

** Chla, Chlb and Chlt mean chlorophyll a, b and total respectively. CAT-catalase activity; APX-ascorbate peroxidase activity; CAR- total carotenoids; FLA-total flavonoids; PHE-total phenolic compounds; TLM-total leaf mass; DM- dry matter; RL-root length; RV- root volume; TRA-transpiration rate; SVTleaf-leaf saturation vapor; Cond- stomatal conductance; CndTotal -total stomatal conductance; WUE-intrinsic water use efficiency; Ls-stomatal limitation value; Pn-net photosynthesis;

CarbE- carboxylation efficiency of Rubisco; C_i and C_a -intercellular and ambient carbon dioxide respectively; T_i and T_a -intercellular and ambient temperature respectively and NDVI-normalized difference vegetative index.

UNDER PEER REVIEW

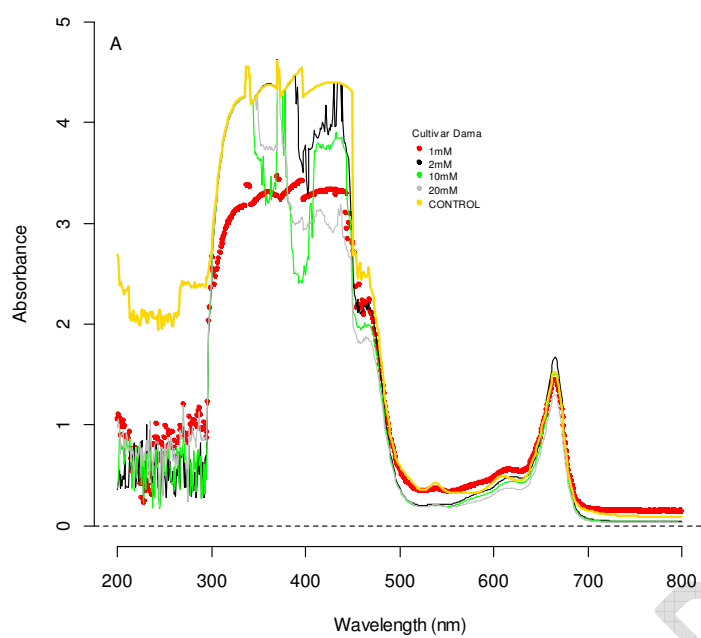
774 **Table 2**

Dama cultivar**											
Potassium amount		0		1		2		10		20	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Pn		6.31	1.6	2.82	2.4	3.32	0.9	5.19	2.6	3.50	2.1
Cond		0.10	0.0	0.04	0.0	0.04	0.0	0.08	0.0	0.04	0.0
Ci		274.05	2.0	299.69	64.4	258.32	25.3	261.44	16.8	249.16	29.6
Trmmol		2.60	0.6	1.04	0.5	1.15	0.2	1.77	1.0	1.07	0.5
TI/Ta		0.57	0.3	1.29	0.2	1.28	0.1	0.97	0.4	1.24	0.2
SVTleaf		4.02	0.1	3.70	0.2	3.85	0.1	3.75	0.1	3.88	0.2
CndTotal		0.10	0.0	0.04	0.0	0.04	0.0	0.07	0.0	0.04	0.0
Ci/Ca		0.70	0.0	0.75	0.2	0.65	0.1	0.67	0.0	0.63	0.1
WUE		61.52	2.3	53.43	32.6	75.70	16.0	71.97	11.3	82.43	14.8
Ls		0.30	0.0	0.25	0.1	0.35	0.1	0.33	0.0	0.37	0.1
CarbE		0.02	0.0	0.01	0.0	0.01	0.0	0.02	0.0	0.01	0.0
Uirapuru cultivar											
Pn		3.25	0.8	3.77	3.1	3.76	2.6	6.67	3.2	5.29	3.0
Cond		0.09	0.0	0.07	0.0	0.08	0.1	0.70	0.7	0.07	0.0
Ci		311.20	26.1	302.73	21.2	301.69	6.1	205.81	129.2	275.08	55.6
Trmmol		2.31	1.0	1.33	0.4	1.66	1.3	4.79	0.9	1.66	0.7
TI/Ta		0.73	0.4	1.15	0.2	0.97	0.6	6.18	8.8	1.01	0.4
SVTleaf		4.01	0.1	3.60	0.4	3.48	0.5	10.97	10.2	3.69	0.3
CndTotal		0.09	0.0	0.06	0.0	0.07	0.1	0.15	0.1	0.07	0.0
Ci/Ca		0.79	0.1	0.76	0.1	0.75	0.0	0.51	0.4	0.70	0.1
WUE		41.96	16.0	51.12	14.5	55.01	9.7	30.97	22.6	67.57	16.3
Ls		0.21	0.1	0.24	0.1	0.25	0.0	0.49	0.4	0.31	0.1
CarbE		0.01	0.0	0.01	0.0	0.01	0.0	0.05	0.0	0.02	0.0

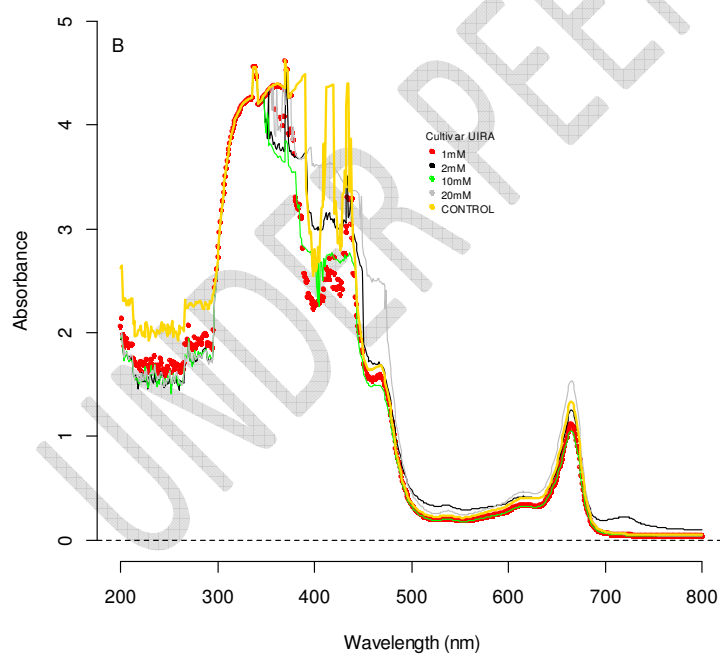
775 ** Net photosynthesis (Pn). stomatal conductance (Cond). intercellular carbon dioxide (Ci).
 776 Transpiration rate (Trmmol). Leaf to air temperature ratio (TI/Ta). Leaf saturation vapor
 777 (SVTleaf). Total conductance to carbon dioxide (CndTotal). Intercellular to ambient carbon
 778 dioxide (Ci/Ca). Intrinsic water use efficiency (WUE). Stomatal limitation value and
 779 Carboxylation efficiency of rubisco (CarbE).

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781 Supplementary Figure 1: UV-Visible scanning spectra



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