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1 **Differential responses of antioxidants and dehydrin expression in two**
2 **switchgrass (*Panicum virgatum*) cultivars contrasting in drought**
3 **tolerance**

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5
6 **Running title**: Switchgrass drought Tolerance and Antioxidant

7

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25

26 **Types of papers:** Original Research Articles

27

28 **Abstract**

29 Drought stress is a major limiting factor for plant growth and development in many regions of the
30 world. This study was designed to investigate antioxidant metabolism and dehydrin expression
31 responses to drought stress in two switchgrass cultivars (drought tolerant Alamo, and drought
32 sensitive Dacotah) contrasting in drought tolerance. The plants were subjected to well-watered
33 [100% evapotranspiration (ET)] or drought stress (30%-50% ET) conditions for up to 24 d in
34 growth chambers. Drought stress decreased leaf relative water content (RWC), increased leaf
35 electrolyte leakage (EL), leaf malondialdehyde (MDA) content in two cultivars, but Alamo
36 exhibited higher leaf RWC level, lower leaf EL and MDA when compared to Dacotah at 24 d of
37 drought treatment. Drought stress also increased superoxide dismutase (SOD), catalase (CAT) and
38 ascorbate peroxidase (APX) activities in two cultivars, Alamo had relatively higher SOD, CAT
39 and APX activities and greater abundance of SOD and APX isozymes than Dacotah at 24 d of
40 drought treatment. Alamo had higher abundance of 55 KDa and 18 KDa dehydrin accumulation
41 than Dacotah under drought treatment. Relative genes expression level of *PvCAT1, PvAPX2,*
42 *PvERD and PvPIP1;5* in Alamo were significantly higher than Dacotah at 24 d of drought
43 treatment. These results suggest that increase in antioxidant enzymes and accumulation of
44 dehydrin were highly related with switchgrass drought tolerance. Antioxidant enzyme activity,
45 isozyme expression and dehydrin abundance could provide a useful screening tool to identify
46 relative drought tolerance in switchgrass cultivars.

47

48 **Keywords:** *Panicum virgatum*, drought tolerance, antioxidant, dehydrin, gene expression

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51

52 **Introduction**

53 Drought occurs in all climates and many parts of the world every year when sufficient water
54 needed to sustain an area is not available, causing significantly impacts on plants growth,
55 development and crop yield [1]. Worldwide losses in crop yields from drought probably exceed
56 the losses from all other abiotic stress combined [2, 3]. Drought stresses cause oxidative stress by
57 contributing to reactive oxygen species (ROS) such as superoxide radical ($O^{\bullet-}_2$), hydrogen
58 peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$) and singlet oxygen ($^1O_2$) [4]. ROS can seriously disrupt
59 normal metabolism through oxidative damage to nucleic acids, lipids, protein and damage
60 membrane function [5]. However, ROS may also serve as a transduction signal during drought
61 stress and improve stress defense mechanisms of plant [6]. ROS levels that are too low or too high
62 affect plant growth and development, maintaining ROS levels within a moderate range is important
63 for plants [7, 8].

64 Plants have developed an antioxidant defense system in response to the high level of ROS [9].
65 There are generally two repairing mechanisms that plants have developed to scavenge free ROS:
66 (i) production of antioxidants or antioxidant enzymes that directly react with and scavenge ROS,
67 including superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidase
68 (POD), and a tocopherol; (ii) production of enzymes that regenerate oxidized antioxidants such as
69 glutathione, glutathione reductase, ascorbate, and ascorbate reductase[10]. Previous studies
70 provided correlative evidence that the enhanced drought tolerance of plant was associated with
71 changes in antioxidant enzymes (SOD, POD, CAT, POX and GR) and maintenance of low H_2O_2

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72 levels [11-13]. It is known that organelles with a highly oxidizing metabolic activity or an intense
73 rate of electron flow, such as chloroplasts, mitochondria, and microbodies, are major sources of
74 ROS, different isoenzymes such as Cu/Zn-SOD, Fe-SOD, Mn-SOD, cytosol APX (cAPX), and
75 microbody APX (mAPX) have been found in different organelles [14]. The antioxidant isozymes
76 could be used as a biochemical marker to study the tolerance of plant to stress. Sen and
77 Alikamanoglu [15] found that two new POX isozyme bands were detected in all drought-tolerant
78 sugar beet mutants compared to the control, and the intensity of Fe-SOD, Cu/Zn-SOD, CAT and
79 APX isozymes were detected at different intensities among the drought-tolerant sugar beet mutants.
80 Recent study found that a new CuZn SOD iszyme OsCSD3 which encoded by LOC_Os03g11960
81 of rice, was up-regulated in response to drought, oxidative stress and salt [16].
82 Dehydrin (DHN) is a multi-family of proteins present in plants that are produced in response
83 to cold and drought stress. DHNs are hydrophilic and reliably thermostable. They are stress
84 proteins with a high number of charged amino acids that belong to the Group II Late
85 Embryogenesis Abundant (LEA) family [17, 18]. DHNs have been divided into five subclasses
86 based on their conserved amino acid sequences: the Y, S and K segments and include YnSKn,
87 SKn, Kn, YnKn and KnS sub-types[19]. It has been reported that the expression of DHN is
88 positively correlated with the tolerance to cold, drought, and salt stress [20]. DHNs play an
89 important protective role during cellular dehydration. The accumulation of DHNs was observed
90 in roots, leaves, coleoptiles, crowns and seeds under drought stress [21].
91 Switchgrass (*Panicum virgatum*) was selected as a model bioenergy crop in the United Sates
92 [22]. To avoid competition of arable lands with food crops, switchgrass will be mainly grown on
93 marginal lands, where millions of hectares of these lands are drought-affected [23]. Two distinct
94 switchgrass ecotypes are generally defined based on morphological characteristics and habitat:

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95 lowland and upland. Lowland ecotypes are mostly tetraploid (2n = 4× = 36) while upland ecotypes
96 are mainly octaploid (2n = 8× = 72) or hexaploid (2n = 6× = 54) [24]. Lowland ecotypes are taller
97 and higher biomass yield, whereas upland ecotypes are shorter and less biomass yield [25].
98 Improving switchgrass yield under drought stress is one of the most important goals of plant
99 breeding. Our previous study have found that switchgrass exhibits a wide range of genetic
100 variability in drought tolerance, Alamo ranked # 4 in drought tolerance and Dacotah ranked # 48
101 among 49 switchgrass lines [26]. However, limited information is available on the gene
102 expressions in conjunction with antioxidant and dehydrin responses and isozyme alterations under
103 drought tolerance in switchgrass. Identifying and understanding the function of antioxidant
104 defense mechanisms are important for developing drought tolerant switchgrass plants. The
105 objectives of this study were to determine whether drought resistance of switchgrass cultivars
106 could be associated with antioxidant metabolism and dehydrin expression levels.
107

108 ## Materials and methods

109 ### Plant materials and culture

110 Two cultivars Alamo and Dacotah were examined in this study. Each switchgrass line was
111 propagated by splitting tillers on Mar, 10, 2014. Five tillers from each line were transplanted into
112 plastic pots (17 cm diam., 20 cm high, with four holes at the bottom for drainage) filled with 3.5
113 kg of a soil and sand mixture (soil:sand = 2:1 v/v, sand: 0.1-1.0 mm diam.). The plants were grown
114 in greenhouse with temperatures of 30±1 ˚C/25±1 ˚C (day/night), a 14-h photoperiod, 75% relative
115 humidity, and with photosynthetically active radiation (PAR) of approximately 500 µmol $m^{-2}s^{-1}$
116 (natural daylight supplemented with fluorescent lamps). The plants were irrigated daily, and
117 fertilizer containing N (Bulldog brand, 28-8-18, 1% ammonia N, 4.8% nitrate N, and 22.2% urea

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118 N; SQM North America, Atlanta, GA) and micronutrients was applied at 0.1 lb. 1000 ft^{-2} every
119 week.
120 After the plants were grown for two months and had reached the E5 developmental stage [27],
121 then moved into a growth chamber for the experiment. The chamber was set at 30/25 ˚C (day/night
122 temperature), 75% of relative humidity, 14 h photoperiod, and PAR at 500 μmol $m^{-2}s^{-1}$. Plants were
123 fertilized once a week, and watered every two days until water drainage occurred at the bottom of
124 the pot at each irrigation.
125

126 **Drought stress treatment**

127 In order to determine the soil water content (SWC) of each pot more quickly, an equation of
128 linear regression between the SWC and volumetric soil moisture content (VWC) was made before
129 the drought treatment. Soil of eight pots was oven dried at 105°C for 48 h to obtain their dry
130 weights (DW). Then we added enough water to each pots, after 1 h when no water leaked from the
131 bottom of the pots, fresh weight of each pot and VWC were measured with a soil moisture meter
132 (model HH2, Delta-T Devices, Cambridge, England) and every three day thereafter. SWC was
133 determined using the formula: SWC (%) = (FW−DW)/DW×100. Then we got an equation of linear
134 regression between the SWC and VWC (Supplemental table 1).
135 Plants were allowed to acclimate to growth chamber conditions for one week before drought
136 treatments were imposed. Each line were randomly assigned to either the control group (n=4),
137 which was kept well-watered (100% ET), or to the drought treatment group (n=4), in which the
138 soil moisture was allowed to progressively decline from 0 d to 24 d. Each pots of drought treatment
139 were weighted every two days and VWC were also collected, then SWC was calculated by the
140 equation we got above (Supplemental table 1), the water needed to add of each pot was calculated

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141 to compensate for 30%-50% ET during the experiment over the 24 d period (Fig. 1).

142

143 **Physiological measurements**

144 Leaf samples were collected for electrolyte leakage (EL) and relative water content (RWC)
145 measurements at day 0, 6, 12, 18 and 24 of drought stress. Leaf tissues were also sampled, frozen
146 with liquid N, and used for antioxidant emzyme and dehydrin analysis.

147 Leaf electrolyte leakage (EL) was measured according to the method of Marcum [28] with
148 some modifications. The top 2nd or 3rd mature leaf blades were excised and cut into 2 cm segments.
149 After being rinsed 3 times with deionized H_2O, 0.2 g leaf segments were placed in a test tube
150 containing 20 mL deionized H_2O. The test tubes were agitated on a shaker for about 24 h and the
151 solution conductivity (C_1) was measured with a conductivity meter (SR60IC, VWR, Radnor, PA).
152 Leaf samples were then autoclaved at 120 ˚C for 30 min, and the conductivity of the solution
153 containing killed tissue was measured once the tubes were cooled down to room temperature (C_2).
154 The relative EL was calculated using the formula: EL (%) = (C_1/ C_2) ×100.

155 Leaf relative water content (RWC) was determined according to the method of Barrs and
156 Weatherley [29]. The Leaf RWC was calculated based on the following formula: RWC= (FW-
157 DW)/ (TW-DW) ×100, where FW is leaf fresh weight, DW is the dry weight of leaves after
158 drying at 85 ˚C for 3 d, and TW is the turgid weight of leaves after soaking in distilled water for
159 24 h at 20 ˚C.

160

161 **Antioxidant enzyme activity**

162 Frozen leaf samples were ground in liquid nitrogen and homogenized in 50 mM sodium
163 phosphate buffer (pH 7.0) containing 2 mM EDTA, 5 mM β-mercaptoethanol and 4% (w/v)

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164 polyvinylpyrrolidone-40 (PVP-40). The homogenate was centrifuged at 12,000×g for 30 min at
165 4 °C. The supernatant was used for assay of the antioxidant enzymes CAT, APX, and SOD.
166 Total SOD activity was determined by measuring its ability to inhibit the photochemical
167 reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries [30]
168 with minor modifications. The reaction solution (1 mL) contained 50 mM phosphate buffer (pH
169 7.8), 0.1 mM EDTA, 13 mM methionine, 65 μM NBT and 1.3 μM riboflavin, and 30 μL SOD
170 extract. A solution containing no enzyme solution was used as the control. Test tubes were
171 irradiated under fluorescent lights 60 $\mu mol \cdot m^{-2} \cdot s^{-1}$) at 25 ºC for 10 min. The absorbance of each
172 solution was measured at 560 nm using a spectrophotometer, and one unit of enzyme activity was
173 defined as the amount of enzyme that would inhibit 50% of NBT photoreduction.
174 The CAT activity was determined using the method of Chance and Maehly[31] with
175 modifications. For CAT, the reaction solution (1 mL) contained 50 mM phosphate buffer (pH 7.0),
176 15 mM H_2O_2, and 30 μL of extract. The reaction was initiated by adding the enzyme extract.
177 Changes in absorbance at 240 nm were read in 1 min using a spectrophotometer (ϵ = 39.4 M^{-1} cm^{-}
178 ^{1}).
179 The APX activity was assayed by recording the decrease in absorbance at 290 nm for 1 min.
180 The 1.5-mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM
181 ascorbic acid, 0.1 mM EDTA, 0.1 mM H_2O_2, and 0.15 mL of enzyme. The reaction was started
182 with the addition of 0.1 mM H_2O_2 [32].
183

184 **Lipid peroxidation**

185 Lipid peroxidation was measured in term of leaf MDA content [33]. A 1 ml aliquot of
186 supernatant was mixed with 4 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid.

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187 The mixture was heated at 100 °C for 30 min, quickly cooled, and then centrifuged at 10000 *g* for
188 10 min. The absorbance of the supernatant was read at 532 nm. The unspecific turbidity was
189 corrected by A600 subtracting from A530. The concentration of MDA was calculated using an
190 extinction coefficient of 155 mM^{-1} cm^{-1}.

191

192 **Antioxidant isozymes**

193 The procedure of protein extraction was the same as for antioxidant enzymes. The extracts (15
194 μL) for SOD and APX were loaded on each gel. Native polyacrylamide gel electrophoresis (PAGE)
195 was performed using a Mini-Protean system (Bio-Rad Laboratories, Hercules, CA) at 4 ^{O}C, 120 V
196 for 90 min, except that SDS was omitted. For SOD and APX, enzyme extracts were subjected to
197 native PAGE with 10% resolving gel and 3% stacking gel and CAT was detected on 7% resolving
198 gel and 4% stacking gel.

199 The total activity of SOD was revealed using the method of Beauchamp and Fridovich[34] with
200 some modifications. The gels were incubated in 50 mM potassium phosphate buffer (pH 7.5)
201 containing 2.5 mM NBT in dark for 25 min. After being washed twice with the same buffer, the
202 gels were soaked in 50 mM potassium phosphate buffer (pH 7.5) containing 30 μM riboflavin and
203 0.4% N,N,N,N-tetramethylethylenediamine 235 (TEMED) in the dark for 40 min. The gels were
204 then illuminated for 10 min with gentle agitation until appearance of enzyme bands and were
205 transferred to 1% (v/v) acetic acid to stop the reaction.

206 The activity of APX was detected using the method of Lopez-Huertas *et al*. [35] with some
207 modifications. The gels were pre-incubated in 50 mM sodium phosphate buffer with 4 mM
208 ascorbate and 2 mM H_2O_2 for 20 min. After briefly being washed with 50 mM potassium
209 phosphate buffer (pH 7.0), the gels were stained in 50 mM potassium phosphate buffer (pH 7.8)

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210 containing 28 mM TEMED and 1.25 mM NBT until the bands were clearly visible. The gels were
211 then washed with distilled water to stop the reaction.
212

213 **SDS-PAGE and western blot**

214 Frozen leaf samples were ground in liquid nitrogen and re-suspended in100 μl 3 × Laemmli
215 buffer containing 16% β-mercaptoethanol.The tissue was then boiled for 10 min and pelleted at a
216 high speed for 10 min.Twenty micro liters of protein extract was applied to and separated on a 10%
217 SDS-PAGE gel. The proteins were blotted to a PVDF membrane using a Bio-Rad Trans-Blot R
218 TurboTM Transfer System. The membrane was blocked with 5% nonfat skim milk in 1 × Tris-
219 saline buffer supplemented with 0.5% Tween20 (1 × TBST). After a brief rinse with TBS, the
220 membrane was incubated in TBS with a dehydrin polyclonal antibody raised from rabbit (Assay
221 Designs) at a dilution of 1:250 for 1.5 h. Next, the membrane was rinsed in TBS containing 0.5%
222 Tween 20 (TBS-T) four times and then placed for 1 h in a solution of goat antirabbit IgG (dilution
223 1:17500) conjugated to alkaline phosphatase (Sigma). The membrane was rinsed in TBS-T four
224 times.The chemiluminescent signals were exposed to autoradiography film (Genesee Scientific,
225 SanDiego, CA) using a Kodak film processor SuperSignal West Pico Chemiluminescent Sbustrate
226 (Prod # 1856136, Thermo Scientific).
227

228 **RNA extraction and quantitative reverse transcription PCR (qRT-PCR)**

229 Total RNA was extracted from 150 mg of leaf tissue using RNeasy plant mini RNA kit (50) (Qiagen,
230 Valencia, CA), and RNA samples were further treated with DNase (Promega, Madison, WI, USA)
231 to eliminate DNA contamination. Integrity of RNA was confirmed with miniaturized gel
232 electrophoresis with the Agilent Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA). cDNA

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233 was synthesized using the DyNAmo cDNA Synthesis Kit (New England Biolabs, Ipswich,MA,
234 USA). The qRT-PCR analysis primer pairs of the corresponding genes were designed according to
235 sequences obtained from the Phytozyme website (Supplemental table 2). Each 20-μl reaction,
236 which contained 15 ng of random hexamers/μl, 10 IU of Moloney murine leukemia virus RNase
237 H^+ reverse transcriptase solution (Thermo Fisher Scientific), and appropriate buffer containing
238 deoxynucleoside triphosphates (dNTPs) and $MgCl_2$ in a final concentration of 5 mM (1×; Thermo
239 Fisher Scientific), was incubated at 25°C for 10 min and at 37°C for 30 min, inactivated at 85°C
240 for 5 min, and finally chilled to 4°C. Two replicate RT reactions were made for each RNA sample.
241

242 **Experimental design and statistical analysis**

243 The experiment was a 4×2 factorial combination (two switchgrass cultivars, and two drought
244 levels: well-watered and drought treatment) in a complete block design (one treatment of one
245 species served as the block) with four replications. All data were subjected to analysis of
246 variance (ANOVA, SAS 8.1, SAS Institute Inc., Cary, NC). The treatment means were separated
247 using Fisher's protected least significant difference (LSD) test at 5% probability level.
248

249 **Results**

250 **Effects of drought stress on physiological parameters**

251 Drought stress reduced leaf RWC, increased leaf EL and MDA regardless of cultivars (Fig. 2, 3
252 & 4). There were significant differences in leaf RWC, leaf EL and MDA in the two cultivars under
253 drought stress conditions when compared to the controls at 18 d and 24 d. Leaf RWC, leaf EL and
254 MDA of Dacotah decreased or increased sharply at both 18 d and 24 d of drought treatments.
255 Alamo had a relatively higher leaf RWC and lower leaf EL and MDA than Dacotah at 12 d, 18 d

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256 and 24 d of drought stress.

257 **Effects of drought stress on antioxidant enzymes activity**

258 SOD activity of both cultivars increased with the increasing duration of drought stress, with a
259 larger extent in drought tolerant Alamo than sensitive Dacotah (Fig. 5). A significant increase in
260 SOD was observed at 18 d and 24 d of drought treatment for two cultivars compred to their control.
261 At 24 d of drought treatment, SOD of Alamo increased to 150.0 μmol min^{-1} mg^{-1} protein, 3.65
262 times of control, SOD of Dacotah increased to 98.3 μmol min^{-1} mg^{-1} protein, 2.64 times of control,
263 SOD of Alamo treatment was 1.53 times higher than Dacotah treatment.

264 CAT activity were progressively enhanced in the two cultivars with duration of drought stress
265 compared to their control (Figs. 6), Alamo had greate CAT activity than Dacotah at 12, 18 and 24
266 d of drought treatment, CAT of Alamo treatment at 24 d was 1.29 times higher than Dacotah
267 treatment at 24 d.

268 APX had the same trend with SOD and CAT (Fig. 7). APX activity of both cultivars increased
269 with the increasing duration of drought stress, the increase of Alamo was significant higher than
270 Dacotah especially at 24 d of drought treatment. At 24 d of drought treatment, APX of Alamo
271 increased to 73.4 μmol min^{-1} mg^{-1} protein, 2.01 times of control, APX of Dacotah increased to
272 49.8 μmol min^{-1} mg^{-1} protein, 1.54 times of control, SOD of Alamo treatment was 1.47 times
273 higher than Dacotah treatment.

274 **Effects of drought stress on antioxidant isozyme**

275 Our activity staining visualized four SOD isozymes (SOD1-SOD4) in two cultivars (Fig. 8).
276 Alamo and Dacotah increased SOD1 abundance under drought stress compared to control, both
277 cultivars increased SOD2-SOD3 abundance under drought stress compared to control, however,
278 only Dacotah decreased SOD4 abundance under drought stress compared to control. Alamo had

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279 relative higher SOD3- SOD4 abundance in response to drought stress compared to Dacotah. Only
280 one isoform of APX was identified in two cultivars (Fig. 9), both cultivars increased APX1
281 abundance under drought stress compared to control, Alamo had relative higher APX1 abundance
282 in response to drought stress when compared to Dacotah.

283 **Effects of drought stress on dehydrin**

284 Western bolot showed that almost no dehydrin accumulation observed in control of both
285 cultivars, however, drought stress significantly increased dehydrin accumulation in both cultivars
286 at 24 d of drought treatment, Alamo had higher abundance of 53 KDa and 18 KDa dehydrin than
287 Dacotah, no dehydrin accumulation of 18 KDa was found in Dacotah (Fig. 10).

288 **qRT-PCR of antioxidant, dehydrin, heat shock protein (HSP) and aquaporin**

289 qRT-PCR results showed that, the level of *PvSOD1*, *PvERD1*, *PvHSP90* and *PvPIP1;5* mRNA
290 were significantly increased in both cultivars under drought treatment compared to their control,
291 the level of *PvCAT1*, *PvAPX2* were significantly increased only in Alamo at 24 d of drought
292 treatment compared to the control instead of Dacotah (Fig. 11). Whereas the increase of *PvSOD1*
293 mRNA level in Dacotah was significant higher than Alamo at 24 d of drought stress, no
294 significant difference in level of *PvHSP90* mRNA between Alamo and Dacotah at 24 d of drought
295 stress.

296

297 **Discussion**

298 Our previous study found that, Alamo ranked #4 in drought tolerance and Dacotah ranked #48
299 among 49 switchgrass lines, Alamo is a representative of good drought tolerant cultivars and
300 Dacotah is a representative of drought sensitive ones [26]. The results of this study agreed with
301 our previous study, showed that drought stress caused cellular and leaf damage to switchgrass as

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302 indicated by decreased leaf RWC, increased EL and MDA. Alamo had higher RWC, *PvPIP1;5*
303 relative gene expression level, lower EL and MDA content than Dacotah at 24 d of drought
304 treatment, indicating that drought stress resulted in more severe damage to cell membranes in
305 Docatah relative to Alamo at 24 d of drought treatment.

306 When plants are subjected to water deficit, drought tolerant cultivars have better Tr and g_s and
307 gas exchange and less oxidative stress, changes in antioxidants genes expression and activities
308 may improve antioxidant defense system and ROS scavenging [36]. SOD is regarded as the key
309 enzyme in the ROS scavenger system because it catalyzes superoxide free radical dismutation into
310 H_2O_2 and O_2, which is the first step of scavenging ROS [37]. APX and POD scavenge H_2O_2 and
311 reduce ROS toxicity. In this study, we choose relative drought-tolerant cultivar Alamo, and relative
312 drought-sensitive cultivar Dacotah to study the drought tolerance mechanisms of drought
313 associated with antioxidant defense. Our results showed that, drought stress increase SOD, CAT
314 and APX in two cultivars especially at 18 d and 24 d treatment. Alamo had significant higher SOD,
315 CAT, APX activities and *PvAPX2*, *PvCAT1* relative gene expression level than Dacotah. R
316 Khanna-Chopra *et al*. [38] found that drought-resistant wheat maintained favorable water relations
317 and lower H_2O_2 accumulation during severe water stress conditions due to systematic increase of
318 antioxidants such as SOD, APX, CAT [39]. Pallavi Sharma *et al*. [11] reported that drought stress
319 caused oxidative damage in rice species as exhibited by an increase in $O^{\bullet-}_2$ and H_2O_2, but drought
320 tolerant rice maintained higher antioxidant enzyme activities (SOD, APX) compared with drought-
321 sensitive rice. These results indicated that antioxidant defense plays an important role in
322 improvement of drought tolerance in plants, drought tolerant cultivars may have greater ROS-
323 scavenging capacity to suppress ROS-induced injury during abiotic stress.

324 Antioxidant isozymes are known to play a vital role in plant drought tolerance. In this study,

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325 antioxidant isozyme diversity of two switchgrass cultivars were investigated to identify band
326 profiles as biochemical marker for the drought tolerance. Drought stress increased abundance of
327 SOD1-SOD3 and APX1 isozyme in two cultivars at 24 d of drought stress, however, Dacotah had
328 relatively lower SOD4 and APX1 abundance when compared to Alamo at 24 d of drought stress.
329 The results of this study indicate that the drought tolerant cultivar Alamo had greater ability to
330 scavenge ROS than the drought sensitive Dacotah. Our results agreed with the previous studies,
331 which showed that various drought-tolerant plants present different isozyme patterns during
332 drought stress conditions [40, 41].

333 Drought induced-accumulation of dehydrin proteins has been associated with drought tolerance
334 in many plant species [42-44]. In this study, two dehydrin polypeptides (53 KDa and 18 KDa) were
335 detected in Alamo under drought stress conditions at 24 d of drought treatment. Interestingly, only
336 one dehydrin polypeptides (53 KDa) was detected in Dacotah, that could be caused by the different
337 drought tolerant ability between the two cultivar under drought stress. Consistently, Alamo had
338 significant higher *PvERD1* relative gene expression level than Dacotah.

339 In summary, drought stress caused damage to switchgrass as evidenced by decreased RWC,
340 increased leaf EL and MDA content due to drought stress treatment. Alamo had relatively higher
341 RWC, lower EL and less MDA content when compared to Dacotah at 24 d of drought stress. SOD,
342 APX and CAT activities increased during drought stress at 18 d and 24 d. Alamo had higher SOD,
343 APX and CAT activities, greater abundance of SOD1-SOD3 and APX1 isozyme than Dacotah
344 under drought stress. Alamo had greater abundance of two dehydrin polypeptides (53 KDa and 18
345 KDa) under drought stress conditions at 24 d than Dacotah. qRT-PCR showed that antioxidant
346 gene *PvAPX2*, *PvCAT1*, dehydrin gene *PvERD1*, and aquaporin gene *PvPIP1;5* instead of heat
347 shock protein gene *PvHSP90* and *PvSOD1*, are the key genes contribute to the drought tolerance

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348 of Alamo, although Alamo had higher SOD activity and SOD isozyme abundance. Our results
349 suggest antioxidant and dehydrin expression are associated with drought tolerance in switchgrass.
350 Our results also suggest that selection and use of cultivars with greater antioxidant enzyme
351 activities and more abundant isozymes under drought stress may be a practical approach to
352 improve switchgrass drought tolerance.

353

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360 and BZ) the Office of Science (BER), U.S. Department of Energy (KC, RB, XZ, and BZ) ".

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461 **Figure legends**

462 Fig.1. Effects of drought stress on soil water content (SWC). Vertical bars indicate LSD value
463 (P=0.05).

464 Fig. 2. Effects of drought stress on leaf electrolyte leakage (EL) of two switchgrass cultivars Alamo
465 and Dacotah. Vertical bars indicate LSD value (P=0.05).

466 Fig.3. Effects of drought stress on relative water content (RWC) of two switchgrass cultivars
467 Alamo and Dacotah. Vertical bars indicate LSD value (P=0.05).

468 Fig. 4. Effects of drought stress on malondialdehyde (MDA) of two switchgrass cultivars Alamo
469 and Dacotah. Vertical bars indicate LSD value (P=0.05).

470 Fig. 5. Effects of drought stress on superoxide dismutase (SOD) of two switchgrass cultivars
471 Alamo and Dacotah. Vertical bars indicate LSD value (P=0.05).

472 Fig. 6. Effects of drought stress on ascorbate catalase (CAT) of two switchgrass cultivars Alamo
473 and Dacotah. Vertical bars indicate LSD value (P=0.05).

474 Fig. 7. Effects of drought stress on ascorbate peroxidase (APX) of two switchgrass cultivars Alamo
475 and Dacotah. Vertical bars indicate LSD value (P=0.05).

476 Fig. 8. Changes in superoxide dismutase (SOD) isoforms of two switchgrass cultivars Alamo and
477 Dacotah under control (well-watered) and drought stress conditions (24 d). Equal amounts (15
478 μL) were loaded in each lane.

479 Fig. 9. Changes in ascorbate peroxidase (APX) isoforms of two switchgrass cultivars Alamo and
480 Dacotah under control (well-watered) and drought stress conditions (24 d). Equal amounts (15
481 μL) were loaded in each lane.

482 Fig. 10. Immunoblots of dehydrin expression in two switchgrass cultivars Alamo and Dacotah
483 under drought stress (24 d).

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484 Fig. 11. Relative transcript levels of *PvAPX2*, *PvCAT1*, *PvERD1*, *PvHSP90*, *PvSOD1* and
485 PvPIP1;5 genes of two switchgrass cultivars (drought-tolerant Alamo and drought-sensitive
486 Dacotah) under well-watered and drought stress (24 d). Each bar represents the mean of three
487 independent replicates with standard error. Different letters of a-c indicates the statistic
488 difference at 0.05 level.
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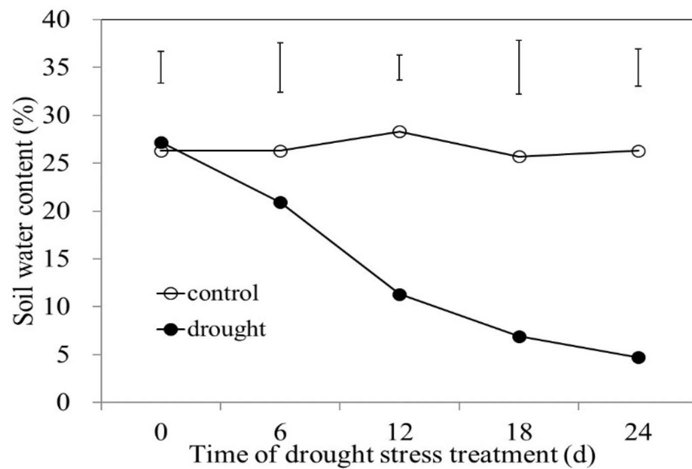
507 Fig.1.



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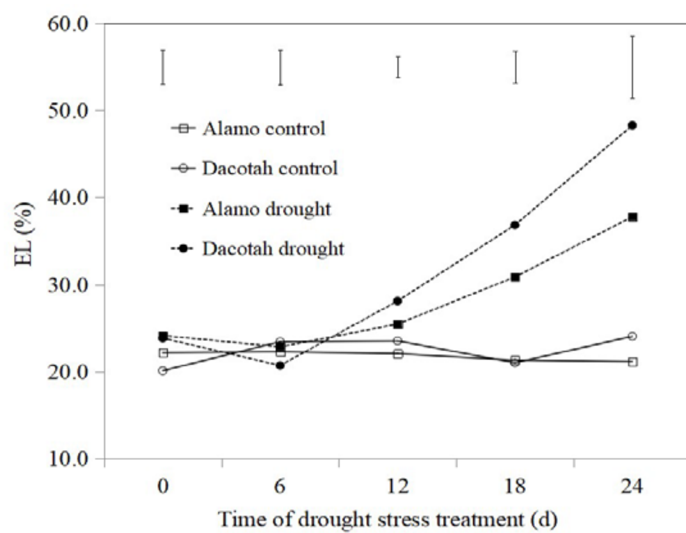

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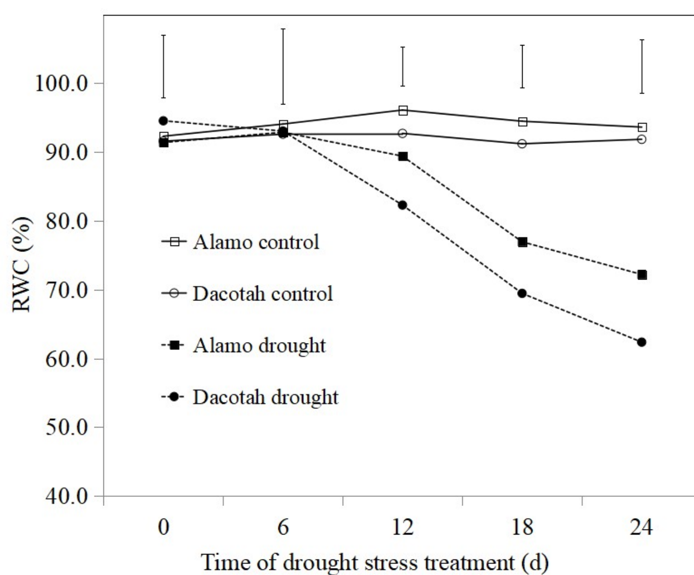
518 Fig.3.



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520 Fig.4.

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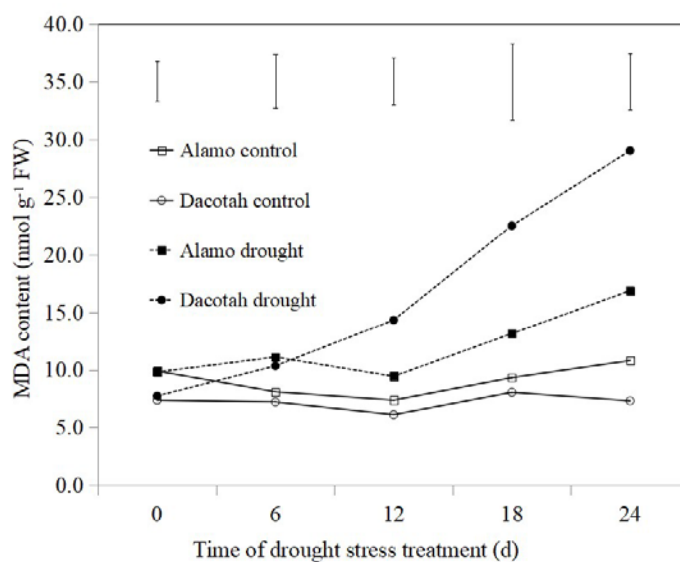

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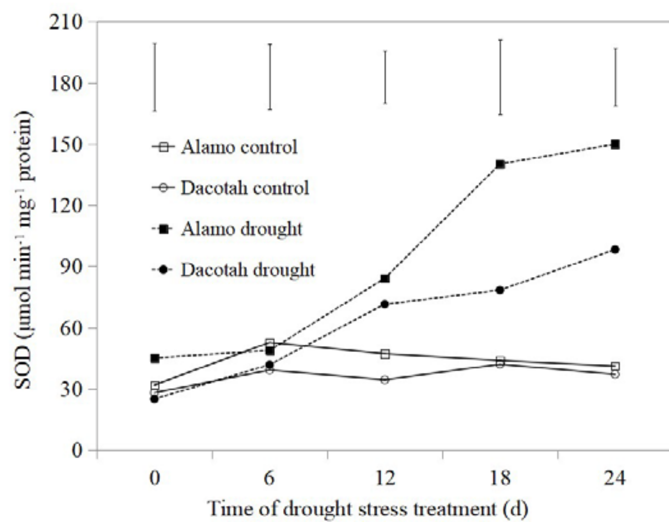
527 Fig.5.



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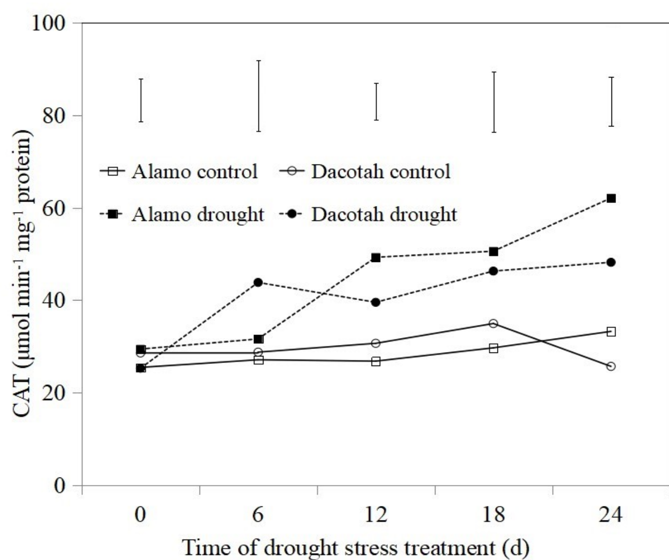

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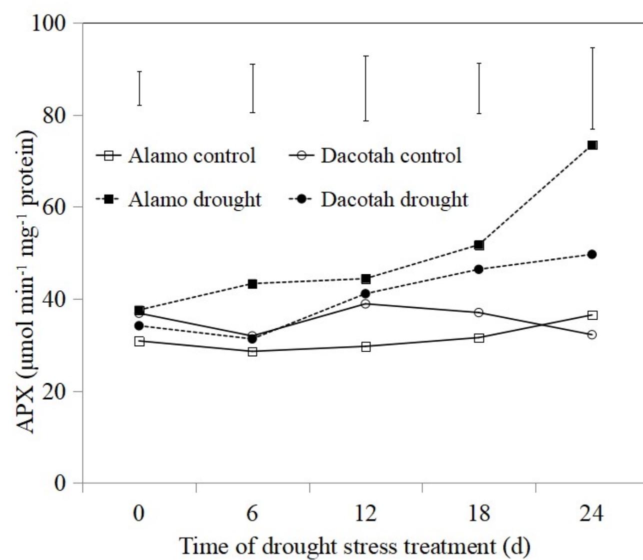
537 Fig.7.



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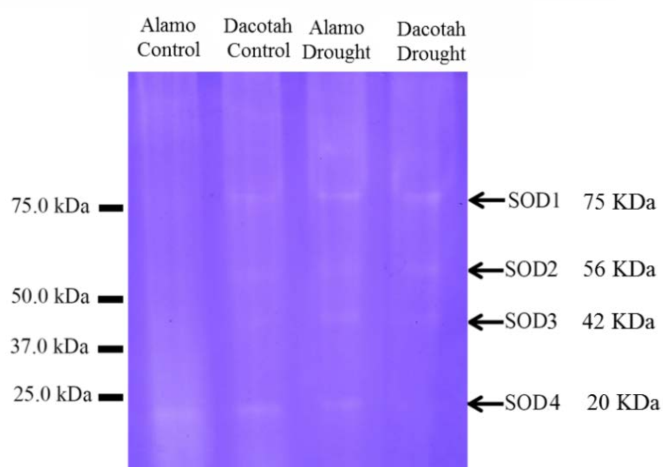

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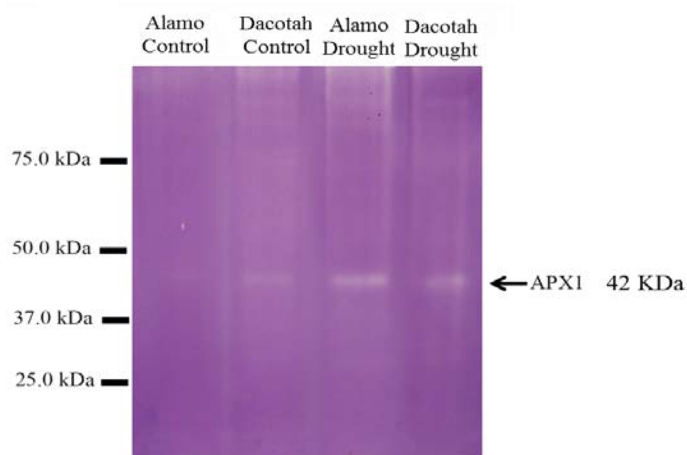
547 Fig.9.



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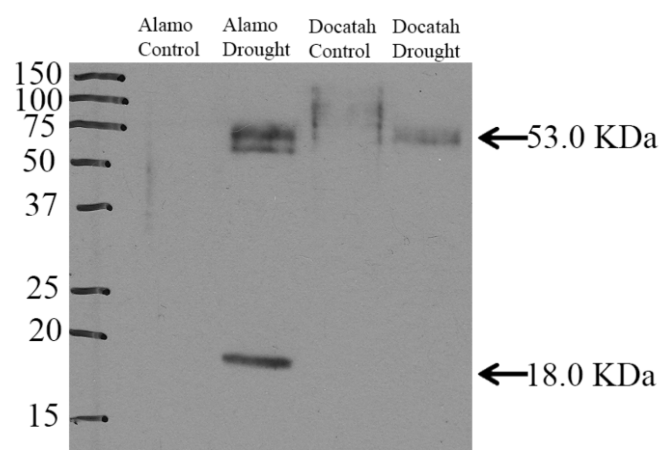

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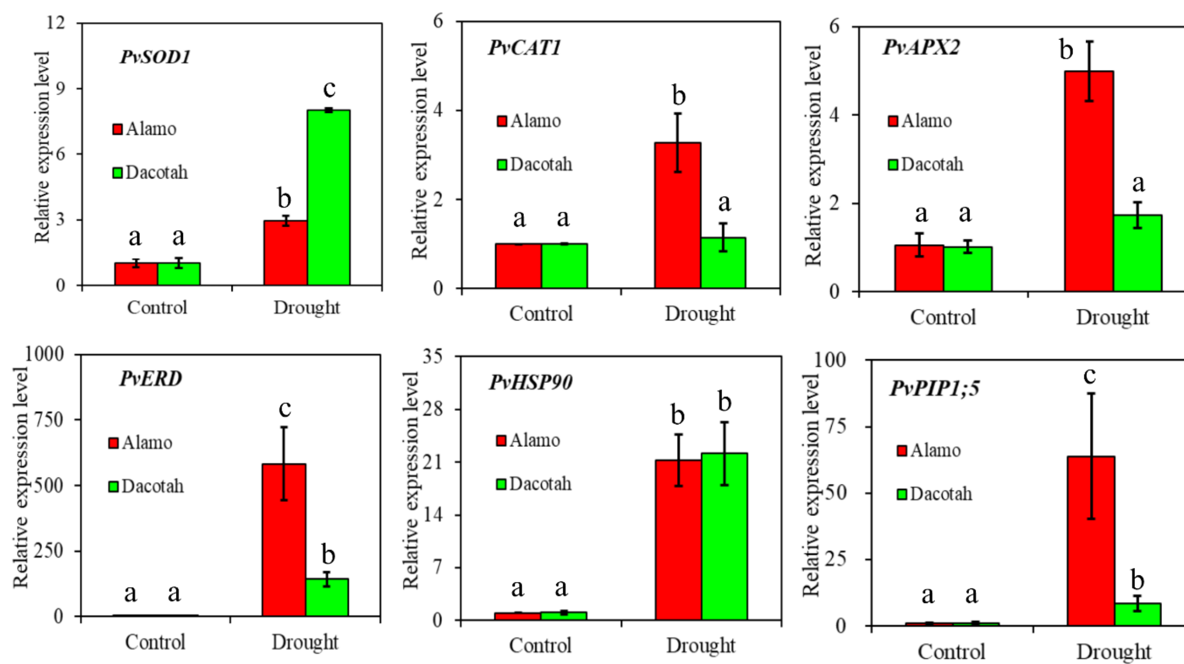
558 Fig.11.



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