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1 ***Physiological and molecular mechanism of tolerance of two maize genotypes***
2 ***under multiple abiotic stresses.***

3

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7

8 ***Running Title: Tolerance mechanism in maize under combined abiotic stresses.***

9 **Abstract:**
10 Abiotic stresses are the major threat to crops regardless of their nature, duration, and frequency,
11 their occurrence either singly, and or combination is deleterious for the plant growth and
12 development. Maize is most important crop largely grown in tropical region in summer rainy
13 season, often face a stress combination of drought and waterlogging. We previously showed under
14 multiple stresses up-regulated leaf proteins of maize plants were involved to enhance the tolerance
15 mechanism of tolerant genotype. Whereas, in susceptible genotypes up-regulated proteins
16 ameliorate to survive the stressful condition. Further to understand the response of roots proteome
17 under multiple stresses was determined using the 2DE technique. The results of the root proteome
18 show the up-regulated proteins of CML49 genotype (tolerant) are involved in enhancing the N
19 content, cell wall remodeling, and acclimatization during the stresses. Up-regulated proteins of
20 CML100 genotype (sensitive) are stressed marker of roots primary and secondary metabolism.
21 However, the root proteome of both genotypes correlates with the leaf proteome (previous).
22 Therefore, the present study and our previous results provide comprehensive insight into the
23 molecular mechanisms of tolerance in multiple abiotic stresses of maize plants.

24

25 **Keywords:** *Multiple stresses, 2-DE, Inbred, Maize, drought x low-N stress, waterlogging x low-N*
26 *stress and Tolerance vs. Susceptibility*

27 .

28 1. Introduction

29 The plants responses to multiple abiotic stresses are unique and shared. The plant responses to
30 stress are dependent on the tissue or organ affected by the stress. The roots are the key organs that
31 perceived stress signals and lead to brought changes at the cellular and molecular levels. The
32 recent climate change predictions model **(IPCC, 2014)** has predicted higher intensity of abiotic
33 stresses which are now becoming more frequent and usually results simultaneously or
34 consecutively. They are more detrimental to crop growth, development, and production. The effect
35 of combined stresses on crops either additive or synergistic that depends on the nature of
36 interactions between the stress factors (**Mittler, 2006; Atkinson et al. 2013; Prasch and**

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37 **Sonnewald, 2013).** Perhaps, plants are capable to be fitted their responses that may be unique and
38 cannot be extrapolated from the response of the plant to each individual stress **(Rizhsky et al.**
39 **2004)**. Maize is the most important cereal crop that is grown at the wide geographical ranges of
40 latitude and longitude of the world. In South Asia, particularly in tropical and subtropical
41 environment maize is a major crop largely cultivated in the summer rainy season, often face a
42 stress combination of drought and waterlogging due to irregular monsoon rains in the region
43 (**Zaidi et al. 2008**). These two stress factors reveal the secondary stress effect of low nitrogen
44 availability. N-uptake affected because of water deficit, while, in waterlogging leaching and de-
45 nitrification of soil nitrogen (**Rathore et al. 1996**). Plants' responses to combined abiotic stresses
46 are a complex mechanism that brought changes at the transcriptome, proteome, and metabolome
47 of the organism. The changes in cellular metabolism and recent advances in 'Omics' technologies
48 have led to new insights into understanding the abiotic stress response of plants. Several workers
49 compared the stress factor at proteome level in different treatments singly or in combined
50 stresses **(Peng et al. 2009; Rollins et al. 2013; Oh and Komatsu, 2015)**. So far, proteomics
51 approaches have broadened our understanding of plant stress response and for improving many
52 physiological traits and in shaping the novel phenotype **(Kosova et al. 2018)**. In our previous
53 work, we have shown under multiple stresses the upregulated proteins of maize leaf were involved
54 to enhance the tolerance mechanism of the tolerant plants whereas, in susceptible plants expressed
55 proteins help to survive the stressful condition. The present work was undertaken in continuation
56 and roots proteome of tolerant and sensitive maize plants were compared in multiple stresses
57 applied concurrently to understand the complex tolerance mechanism at the molecular level.
58 Besides, the physiological analyses of the two inbred maize plants also completed to understand
59 the differential adaptability of the two genotypes to multiple stresses.
60

61 ## 2. Materials and Methods

62 ***Plant Material and Growth conditions***

63 In the present work two maize inbred (CML49 & CML100) genotypes were selected that showed
64 different adaptability to various abiotic stresses applied simultaneously. The selected two
65 genotypes (CML49 and CML100) with 80 pots each were grown in natural conditions in
66 greenhouse up to 30 days. Thirty days after sowing (DAS) were subjected to multiple abiotic
67 stress treatment, first 40 pots of each genotype were exposed to a drought (drought x low-N) for
68 10 days, while 40 control pots were supplied with full nutrient and water. After re-watering for
69 two days normally, the same plants were exposed to waterlogging (waterlogging x low-N) for up
70 to 7 days, to maintain the water level 2-3cm above the soil surface of the pots plants were watered
71 day and night. The roots samples from 3-stressed and 3-control replicates plants of each genotype
72 were kept on the last day of combined stresses for physio-biochemical analysis and extracting

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73 proteins. Roots samples were quickly frozen in liquid nitrogen after removing from the plant and
74 then kept at -80^0 C for further analysis.
75

76 ***Determination of Morphological parameters***

77 To measure fresh/dry shoot and roots weights, seedlings were pulled out of the soil and roots
78 carefully washed to remove soil particles and roots fresh weight were measured. For fresh shoot
79 weight, the plants were cut at the stem base and measured fresh weight on electronic balance.
80 Then stem and roots oven dried (75°C) for 48 h, for dry weight measurements by means of
81 electronic balance. Total dry weight was calculated by adding the dry weight of shoot and root.
82

83 ***Determination of Physiological parameters***

84 Total protein was extracted by homogenizing the roots, first in liquid nitrogen, then in 4 volumes
85 of 125 mM Tris-HCl buffer, pH 8.8, 1% (w/v) SDS, 10% (w/v) glycerol, and 1 mM PMSF. The
86 homogenate was centrifuged for 10 min at 15000g at 4^{0}C, and the protein content of the
87 supernatant was determined using Bio-Rad Protein Assays Dye Reagent Concentrate and bovine
88 serum albumin as standard. Chlorophyll was extracted by homogenizing the leaf, using mortar and
89 pastel in 4 volumes of 80% (v/v) precooled acetone. The homogenate was centrifuged for 20 min
90 at 12000g at 4 ^{0}C, and the chlorophyll content of the supernatant was measured at 663 and 647 nm
91 in a spectrophotometer (Hitachi U2910). In drought x low-N stress after one week, leaf samples
92 were collected from the second leaf for leaf RWC determinations. The relative water content
93 (RWC) was calculated by using the formula as: RWC% = FW-DW/TW-DW x 100%.
94

95 ***Determination of Biochemical parameters***

96 In vivo assay of NR activity of roots was estimated by following the procedure, **Hageman and**
97 **Hucklesby (1971).** The roots were cut and placed in ice-cold incubation medium containing
98 3.0 ml of 0.2 M potassium phosphate buffer (pH 6.8), and 3.0 ml of 0.4 M KNO3 solution.
99 The tubes were evacuated, with a vacuum pump and then incubated in a water bath at 33°C for
100 one hour under dark conditions. At the end of incubation period, tubes were placed in boiling
101 water bath for 5 min to stop the enzyme activity and the complete leaching of the nitrite in the
102 medium. The calibration curve was prepared using sodium nitrite solution. The enzyme activity
103 was expressed as $\mu mol\ NO^{-2}\ gram\ fresh.weight^{-1}\ hour^{-1}$.
104

105 ***Statistical analysis***

106 The data (Fresh and dry shoot/root weight, total protein content, and chlorophyll content, NR
107 activity in leaf and root, Nitrite content and RWC) were analyzed by one-way analysis of variance
108 (ANOVA) and the means were compared using the post hoc test of Tukey's . All the data were

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109 computed using SPSS version 17. The means of three biological replicates were presented of all
110 the traits.
111
112
113
114 ***Protein Extraction***
115 Total soluble proteins were extracted from three biological replicates of roots from control and
116 stressed of each genotype separately. Approximately, 5 g of roots was ground with liquid nitrogen
117 and homogenized in 10 mL of 0.5 M Tris-HCl, pH 7.5, lysis buffer containing 0.7 M sucrose, 50
118 mM EDTA, 0.1 M KCl, 10mM thiourea, 2mM PMSF and 2% v/v -mercaptoethanol. Then
119 saturated phenols 10mL of Tris, pH 8 was added. After mixing for 30 min the phenolic phase was
120 separated by centrifugation and rinsed with another 10 mL of lysis buffer. Protein was precipitated
121 overnight at –20^{0}C after adding 5 volumes of methanol containing 0.1 M ammonium acetate. The
122 pellet recovered by centrifugation was rinsed with cold methanol and acetone, air-dried and re-
123 suspended in 340 µl rehydration buffer. The protein content of samples (supernatants) were
124 quantified by the method of Bradford **(Bradford 1976)** using bovine serum albumin (BSA) as a
125 standard.
126
127 ***2DE-Gel Electrophoresis***
128 Three biological and two technical replicates were performed form each genotype (CML49 and
129 CML100) treatment and control plants. For first dimensional electrophoresis isoelectric focusing
130 (IEF), was conducted by using 18cm strips (pH 3-10, linear gradient) in an *Ettan IPG phor IEF*
131 *System* (*GE* Amersham) the total proteins supernatant from the roots tissue having 300 ug proteins
132 in a buffer (9 M urea, 4% w/v CHAPS, 2% Triton X, 100 mM DTT and 2% v/v IPG buffer pH 3–
133 10 *(GE Healthcare)* was applied to the strips. The strips passively rehydrated for 2 hour and for
134 next 10 hour active rehydration at 50V. The IEF was performed according to the following
135 procedure: 250V-30min, 500V-30min, 10,000V gradient-3 hours, (until it reaches 70,000V hours)
136 and finally 500V hold for 20min. After IEF, the strips were equilibrated for reduction and
137 alkylation in equilibration buffer containing 6M urea, 1M Tris-HCl pH-8.8, 10% SDS, 30%
138 Glycerol, 1% bromophenol blue. Initially the strips were treated with EB-1 (10mL EB+100mM
139 Dithiothreitol) for 30 min, thereafter with EB-2 (EB+ 55mM Iodoacetamide) for 30 min. For
140 second dimension the strips were loaded onto a 10% SDS-PAGE Gel *(Ettan Dalt Six Gel*
141 *Electrophoresis Unit from GE)*, The molecular marker ladder of 250 KD-10 KD was applied on
142 one side of the gels and strips were sealed with agros gel, after electrophoretic run at a currant of
143 10 mA per gel for 15 min, they adjusted to 30 mA till the dye reaches at the bottom of the plate.
144 After 2DE, the gels were fixed in 10% methanol and 7% acetic acid for overnight .and silver
145 stained. The gels were scanned at 300 dpi to obtained digital images (TIFF files) using image

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146 scanner *(Epson Expression 11000XL)* and spots detection was performed with Image Master 2D
147 Platinum Software *(GE Healthcare)*. The spots were matched and detected by choosing a gel as
148 the reference gel semi-automatically (manual correction and with default spot analysis setting in
149 the software). Spots were normalized as the percentage of total spot volume that were present in
150 all gels. The spot volume data was analyzed and Student t-test ($p<0.05$) was performed to verified
151 statistically significant changes in spots. The molecular weight of the protein spots was calculated
152 using the standard ladder applied to the gels, though pI of the protein spots determined with
153 manufacturer's instruction *(GE Healthcare)*. Spots were consider reproducible when they were
154 also present in replicates gels. Differentially expressed protein spots with 1.5 or more fold
155 variation in abundance were selected for mass spectrometry identification.
156
157 ***Protein Digestion and Identification using MALDI-TOF/MS***
158 Protein spots were excised manually from each set of gel, excised protein spots were de-stained in
159 400 μL of a 50% (v/v) acetonitrile, 25 mM NH4HCO3 solution for 30 min at room temperature.
160 The procedure was repeated twice. The solution was discarded and 200 μL of pure acetonitrile was
161 added for 5 min and dried under vacuum. The spots were reduced with 10mM DTT and alkylated
162 with 50mM iodoacetamide (Bio-Rad). The proteins were then incubated for 30 min on ice in the
163 presence of 10 μL of 20 ng/μL trypsin (Trypsin V5280, Promega), followed by 16 h at 37 ^{0}C. The
164 peptides were extracted with 30 μL of a 5% trifluoroacetic acid solution. Then the extract was
165 dried under vacuum and solubilized in 0.1% trifluoroacetic acid. The digested fragments were
166 analyzed on MALDI-TOF analyzer (Autoflex II; Bruker Delatonics). Protein identification was
167 performed using the Mascot software database (MSDB). The following parameters were used for
168 database searches: taxonomy- Viridiplantae; enzyme- trypsin; fixed modifications-
169 carbamidomethyl cysteine; variable modifications- oxidized methionine; peptide mass tolerance-
170 0.25 Da; peptide charge- 1 H+. Protein identifications were considered with a Mascot score >40
171 and sequence coverage of at least 25%.
172

173 ## 3. Results

174 ***Combined effects of drought, waterlogging stress and low-N stress on two maize genotypes***
175 The various morpho-physiological parameters measured in two maize genotypes under combined
176 abiotic stresses shows significant reduction in stressed plants compared to controls of both
177 genotype, but the parameter decreases were more obvious in CML100 compared with CML49
178 **(Table 1)**. Similarly, drought stress significantly reduced leaf RWC greater in CML100 (54.33%)
179 compared with CML49 (39.64%) respectively. The decrease in chlorophyll content was less
180 obvious in CML100 (32.30%) and CML49 (34.25%) in drought stress. However, under
181 waterlogging stress decreased in chlorophyll content in CML100 (44.56%) was much higher than
182 CML49 (11.69%), thus under waterlogging stress CML49 shows steady chlorophyll content.

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Similarly, the difference in roots total protein content was less between CML49 (53.18%) and CML100 (52.04%). Nitrate Reductase, the main enzyme which plays a key role in nitrogen fixation responds to many environmental factors. Subsequently, low-N stress was applied to the plants (25% N of the recommended amount of urea), besides nutrients are less available under drought, or waterlogging conditions that may be low water availability, leaching or denitrification. Therefore, NR activity of roots was measured in both inbred plants after combined stresses and the results shows, Nitrate Reductase activity in CML49 was higher (2.019±0.236 $\mu mol\ NO^{-2}\ g\ fr.wt^{-1}\ hr^{-1}$) relative to CML100 (0.673±0.180 $\mu mol\ NO^{-2}\ g\ fr.wt^{-1}\ hr^{-1}$.), in CML49 the percent increase in NR activity was (>60%) high and significant than CML100 respectively. On the basis of physiological evaluation, we can say that CML49 shows more stable performance under combined stresses and could be categorized as 'tolerant'. Whereas, CML100 was more stress sensitive and exhibited reduction in various morpho-physiological and biochemical parameters, hence consider as 'sensitive' inbred. Therefore, both genotypes have differential adaptability under combined stresses.

2-DE gel analysis of roots proteome of two genotypes

The roots proteomic study was accomplish after exposing the two genotypes to combined stresses. The 2 DE gels of roots CML49 and CML100 were compared to their controls and differentially up-regulated protein spots were analyzed by MALDI-TOF by using Mascot database search for identification. Over 807 spots, were identified from each silver-stained gel of both genotype. The number of spots were higher in CML49 (418) compared to treated gels of CML100 (327**)**. Among differentially up-regulated protein of spots of roots that exhibited quantitative changes greater than 1.5 fold variation in abundance, were selected for detailed analysis by MALDI-TOF. The total sixty three (63) spots up-regulated in CML49 **Fig 1,** whereas the up-regulated spots in CML100 was forty (40) **Fig 2**. The list of identified protein spots by Protein mass fingerprinting from both genotypes was shown **Table S1** (Supplementary) functional categorization of the proteins was done according to **M. Bevan et al (1998)**.

4. Discussion

Plant growth and development depends on a vigorous root system, besides anchorage, roots plays key role in nutrient and water uptake. Our roots proteomics study shows the up-regulation of 'Expansin protein' in CML49 genotype. The activity of Expansin is associated with cell wall loosening in growing cells **(Lee et al. 2001)**, its localized expression in meristems and growth zones of roots, and also stem and leaf primordial **(Reinhardt et al. 1998)** was observed. Expansin, strongly regulated by water stress deficiency **(McQueen-Mason, 2004)**, similarly, in soybean, *GmEXPB2*, a gene that encodes a β-expansin (*EXPB*), also induced by mild water deficiency **(Guo et al 2011)** might involved in the response of the root system architecture. In maize roots, its

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220 activity increased by 4-fold (apical region) and by 2-fold (basal region) under low water potential,
221 may be related to the higher expression of expansin encoding genes in the root apical zone,
222 corresponding to maintaining elongation **(Wu et al. 1996; Wu et al. 2001)**. In contrast, other
223 proteomic study showed in soybean seedlings expansin-like B1-like proteins were highly
224 regulated in response to flooding stress and involved in cell wall metabolism **(Nakamura and**
225 **Komatsu, 2013).** Cyanoalanine hydratase (E.C. 4.2.1.65) is an enzyme involved in the cyanide
226 detoxification process. One of the protein spot *Cynate hydrates (24)* up-regulated in the roots of
227 CML49. Higher plants produced CN by six different mechanisms, including ethylene (ET) and
228 camalexin biosynthesis, **(Yip and Yang 1988; Sanchez-Perez et al 2008)**. Higher plants
229 maintaining cyanide homeostasis, through cyanide detoxification process that converts cyanide to
230 β-cyanoalanine (β-Cas pathway), which is then converted to Asn, Asp, and ammonia by NIT4
231 class nitrilases, thus recycle of nitrogen within the plant (**Piotrowski 2008).** Further, reports have
232 shown that plants are capable of utilizing cyanide as a supplemental source of nitrogen (**Ebbs et al**
233 **2003; Siegien and Bogatek 2006).** Higher NR activity of stressed roots and up-regulation of
234 cynate hydrates proteins might attributed for tolerance to CML49 plants. The *tRNA (guanine-N (7)*
235 *methyltransferase (223)* that catalyze the formation of N7-methylguanine at position 46 (m^7G46)
236 in tRNA. Also, several structurally different modified nucleosides are present in different tRNAs,
237 at different position that have a common function to improved reading frame maintenance
238 **(Urbonavieius et al 2001**). Similarly, **Lang et al (2010)** associated it with drought tolerant as
239 drought being a complex trait, many genes are involved it's tolerance. Therefore, the up-regulated
240 proteins played crucial role in tolerance mechanism of CML49 under various combined stress
241 conditions. These results are well coherent with our leaf proteome study (Previous). On the
242 contrary, CML100 shows higher activity of *Alcohol Dehydrogenase (95)* Alcohol dehydrogenase.
243 (Fermentative enzyme that is highly conserved across species) several findings have confirmed
244 that Alcohol dehydrogenase expression increased 6 h after flooding and decreased 24 h after
245 draining water. Root apical meristem showed the strong induction of Adh2 expression in RNA and
246 protein levels under flooding on the contrary, osmotic, cold, or drought stress has no expression of
247 Adh2 genes (**Komatsu et al. 2011***)*. Further, at early vegetative stage, soya bean roots under
248 waterlogging stress shows high up-regulation (9–16-fold) of alcohol dehydrogenase (Adh) protein
249 and may involve in continuing glycolysis **(Iftekhar Alam et al. 2010).** Later, **Ismond et al (2013)**
250 showed the role of ADH in recycling of NADH to NAD+, for the continuation of glycolysis
251 pathway and is the only energy source under oxygen deprivation condition. However, it had no
252 effect on flooding survival **(Ismond et al 2003).** The induction of *Caffeic acid 3-O-*
253 *methyltranferase (79),* protein in CML100 roots and similar induction (CCoAOMT enzyme) was
254 observed under salinity stress in the roots of Arabidopsis and rice **(Salekdeh et al 2002; Lee et al**
255 **2008).** The up regulation of *Sucrose Phosphate Synthase (117)*, shows that in photosynthetic and
256 non-photosynthetic tissues SPS is regulated through metabolites and protein phosphorylation

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257 **(Reimholz et al 1994).** Likewise in wheat, SPP gene family constitutively expressed in source
258 (leaves and germinating seeds) and sink tissues (developing seeds and roots) **(Lunn 2003).**
259 Recently, **Gloria et al (2017***)* observed the expression of sps gene; AtSPS1F, AtSPS2F and
260 AtSPS3F in the columella roots of A thaliana plants hence, the findings support that sucrose
261 synthesis can occurs in the columella cells, and transcriptional analysis shows AtSPS2F and
262 AtSPS4F activated in response to osmotic stress. Similar studies by **Kaur et al (2007)** under water
263 deficit condition in tolerant cultivar and sensitive cultivar of wheat seedlings exhibited the Sucrose
264 synthase activity was lower in the shoots and roots of stressed seedlings of tolerant cultivar. These
265 findings in agreement of our results and suggest the possible role of SPS protein of CML100 plant.
266
267 **5. Conclusion**
268 The Physio-biochemical studies shows the response two genotypes differed in various combined
269 stresses. The CML49 was tolerant and acclimatized to the various abiotic stresses, whereas,
270 CML100 shows sensitive behavior towards multiple stresses. The present study shows up-
271 regulated proteins of roots of CML49 plants under multiple stress conditions are involved in cell
272 wall remodeling, cyanide detoxification, and drought tolerant mechanism. Thus, roots proteins
273 were in alliance with leaf proteome of tolerant inbred. In same way, up-regulated proteins of
274 CML100 plants involved in root primary and secondary metabolism to protect against the stresses
275 and biomarker of anoxia stress, the result was coherent with leaf proteome. In our previous work
276 we have shown that **(Suphia R, 2019)**, up-regulated leaves proteins of tolerant and susceptible
277 maize plants in response to multiple abiotic stresses were involved in enhancing tolerance
278 mechanism of tolerant inbred and up-regulated proteins of susceptible inbred help to survive the
279 stressful conditions. Therefore, the study provides the comprehensive analysis of tolerance
280 mechanism that can be further utilized to develop the climate resilient crop varieties.
281
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287
288 **Conflict of Interests**
289 All authors declared there is no "conflict of Interest".
290
291 **Availability of data and material**
292 ***Table S1:*** The following files below contains the detailed roots Proteomics data (Supplementary
293 data files)

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294

295 **Authors Contributions**

296 Suphia Rafique has contributed to the study conception and design. Material preparation, data
297 collection and analysis and interpretation, writing the manuscript. The Mentor of the Project is
298 [Professor M Z Abdin]. The first draft of the manuscript was written by [Suphia Rafique] and all
299 authors read and approved the final manuscript

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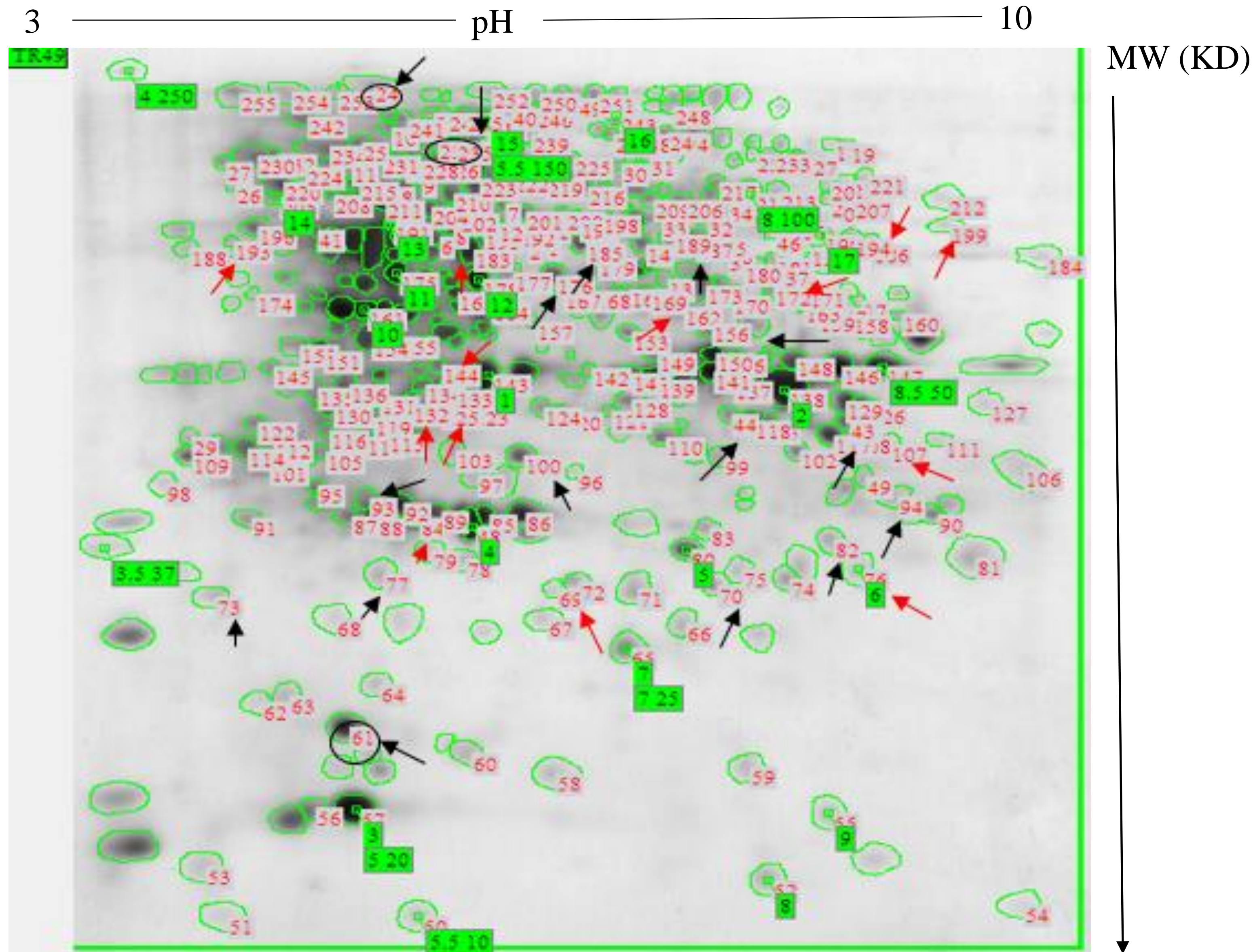
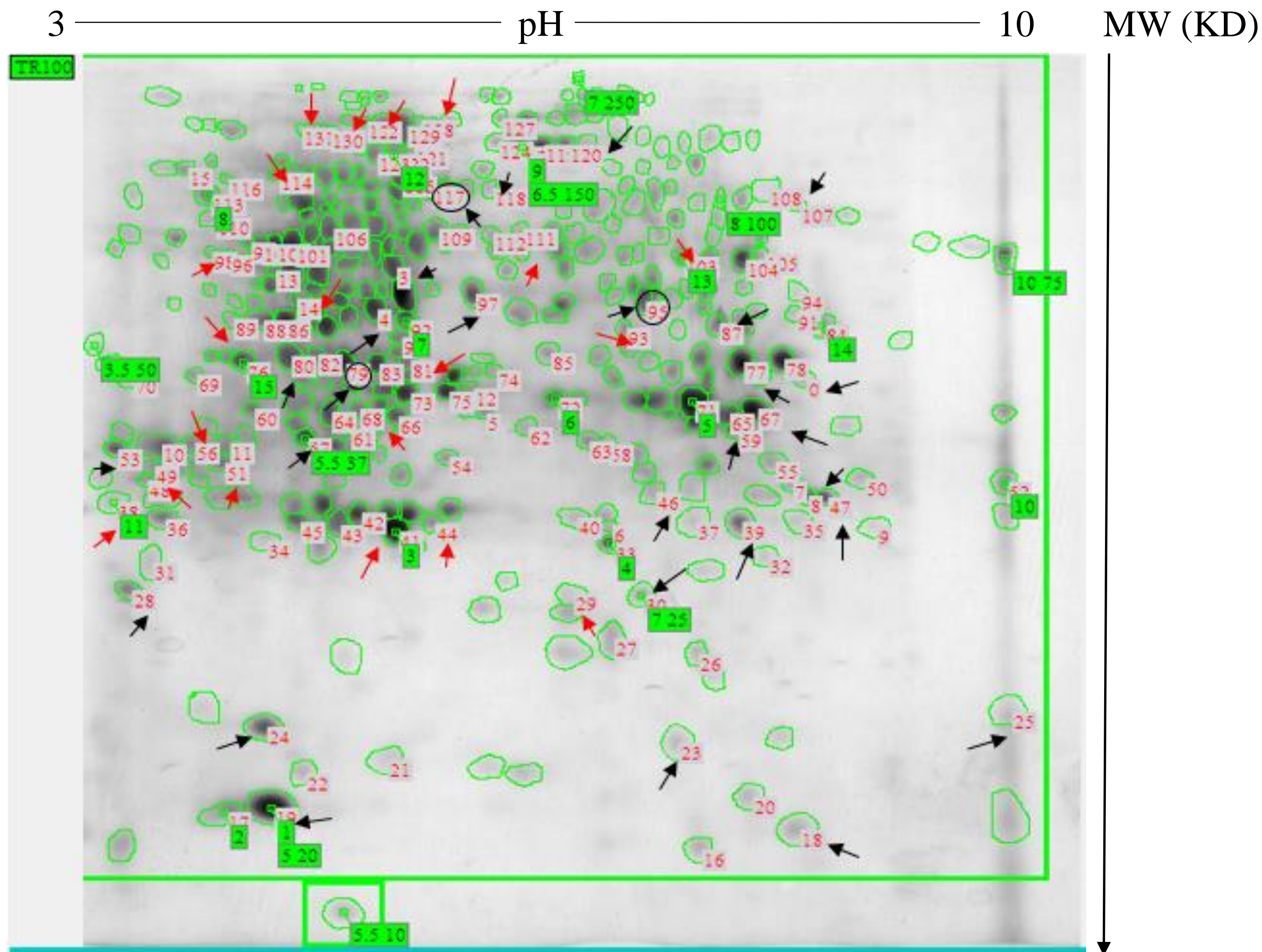
Fig 1. 2-DE representative gel from CML49 (stressed roots)



2DE gel: Fig 1 shows the representative gel of inbred CML49 roots. Black arrows- up regulated proteins spots. Red arrows- down regulated proteins spots. Spots with circle- represented the identified proteins spot by MALDI-TOF

Fig 2. 2-DE representative gel from CML100 (stressed roots)



2DE gel: Fig 2 shows the representative gel of inbred CML100 roots. Black arrows- up regulated proteins spots. Red arrows- down regulated proteins spots. Spots with circle- represented the identified proteins spot by MALDI-TOF

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Physiological and molecular mechanism of tolerance of two maize genotypes under various combined abiotic stresses.

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Abstract

Purpose: Abiotic stresses are the major threat to crops regardless of their nature, duration, and frequency, their occurrence either singly, and or combined. Maize is the most important crop largely grown in the tropical region in the summer rainy season, often faces a stress combination of drought and waterlogging. We previously showed under multiple stresses up-regulated leaf proteins of maize plants are involved to enhance the tolerance mechanism of tolerant genotype and up-regulated proteins in susceptible genotypes help to survive the stressful condition.

Method: To understand the response of two maize genotypes to various combined stresses, physio-biochemical analysis was done. Further, to understand the molecular aspects roots proteomics study was achieved using the 2DE technique.

Results: The physio-biochemical analysis of the two inbred shows a difference in adaptability to the various combined stresses, CML49 shows tolerance and CML100 was sensitive towards the various stresses. The root proteome shows the up-regulated proteins of CML49 genotype are involved in enhancing the N content, cell wall remodeling, and acclimatization during the stresses. Up-regulated proteins of CML100 genotype were stressed marker of roots primary and secondary metabolism.

Conclusion: However, the root proteome of both genotypes correlates with the leaf proteome (previous). Therefore, the present study and our previous results provide comprehensive insight into the molecular mechanisms of tolerance in combined abiotic stresses of maize plants.

Keywords: *Combined stresses, 2-DE, drought x low-N stress, waterlogging x low-N stress and Tolerance vs. Susceptibility; Inbred; Maize*

Table 1. The various parameters measured in control and stressed plants of CML49 and CML100 inbred genotypes under combination of stresses.

Traits	Genotype CML49		Genotype CML100	
	Control	**Treated**	**Control**	**Treated**
Shoot Fresh weight (g/plant)	73.28±14.2	95.65± 36.2	45.88±7.46	21.46±10.23
Root Fresh weight (g/plant)	14.26±4.31	12.39±5.258	9.486±3.95	2.981±2.53
Total Fresh weight (g/plant)	82.3196±9.229	108.040±19.173	55.3637±6.6837	24.4448±6.202
Shoot dry weight (g/plant)	20.4659±3.152	25.025±5.324	17.6963±5.153	7.88125±1.556
Root dry weight (g/plant)	3.4663±0.983	2.3201±0.8688	3.856±1.022	0.2185±0.897
Total dry weights (g/plant)	23.932±3.511	27.345±6.169	21.552±5.559	8.0998±1.579
Total proteins (µg g^{-1} FW)	32.19±7.60	15.07±1.56	14.52±1.86	6.963±3.16
Roots NR Activity (µmol NO^{-2} g $fr.wt^{-1}$ hr^{-1})	2.696±2.53	2.019±0.709	1.936±0.565	0.673±0.541
Leaf RWC (%)	95.552±3.59	57.674±6.487	89.804±4.86	41.006±13.8
Chlorophyll content (µg/g/ fresh weight) (DxLN)	2.467±0.9068	1.622±0.193	1.941±0.6177	1.314±0.1145
Chlorophyll content (µg/g/ fresh weight) (WxLN)	1.462±0.111	1.291±0.9758	2.098±0.884	1.163±0.639

The values represent the Means ± SDs. ($p<0.05$)

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