Anais da Academia Brasileira de Ciências (2018) 90(2): 1493-1501
(Annals of the Brazilian Academy of Sciences)
Printed version ISSN 0001-3765 / Online version ISSN 1678-2690
http://dx.doi.org/10.1590/0001-3765201820170632
www.scielo.br/aabc | www.fb.com/aabcjournal

ABA regulation of post-germination desiccation tolerance in wheat cultivars contrasting in drought tolerance

SATINDER KOUR and VIKRAMJIT K. ZHAWAR

Department of Biochemistry, College of Basic Sciences & Humanities, Punjab Agricultural University, 141004, Ludhiana, India

Manuscript received on August 14, 2017; accepted for publication on November 16, 2017

ABSTRACT

Post-germination desiccation tolerance (DT) was studied in two wheat cultivars. Effect of pretreatment of abscisic acid (ABA)/ osmotic/ salt/ heat stress was also studied. One day (d)-old seedlings of wheat cultivars PBW 644 (drought tolerant) and PBW 343 (drought susceptible) were exposed to ABA/stress treatment for next 1 d, desiccated for 4 d and subsequently rehydrated for 4 d. Biomass, protein, water, protein carbonyls (oxidative toxicity) and nitric oxide (NO) levels were measured in 2 d (treated), 6 d (desiccated), 10 d (rehydrated) seedlings. Vegetative reactive oxygen species (ROS)/ NO-pathways were studied under normal condition and ABA supply by supplying ROS/NO scavengers. Desiccation caused water loss and increased oxidative toxicity. PBW 644 showed very low level of toxicity but higher loss of water under desiccation. ABA/ stress pretreatment further reduced water level under desiccation and reduced biomass upon rehydration in PBW 644 only. On the other hand, PBW 343 did not show higher decrease of water but showed high toxicity under desiccation where ABA/stress pretreatment improved this response by increasing biomass upon rehydration. This indicated that PBW 644 used metabolic arrest under desiccation for survival while PBW 343 used growth promotive mode. ABA/ROS/NO-pathways were operational in both cultivars.

Key words: abiotic, abscisic acid, nitric oxide, post germination desiccation tolerance, wheat.

INTRODUCTION

Desiccation tolerance (DT) refers to the ability of a cell to endure loss of all or almost all of its water without irreversible damage. Vegetative DT is not common in angiosperms except resurrection plants (Gaff and Oliver 2013, Lyall et al. 2014). Though DT is rare in angiosperms, DT is common in their reproductive tissues like seed embryos and pollens. Majority of angiosperm species produce orthodox seeds (desiccation tolerant) while some produce recalcitrant seeds (desiccation sensitive). Orthodox seeds acquire DT in the development process during seed maturation and lose DT during germination. Hence studying the dehydration response of seeds during development and/or germination is a common approach for the study of DT (Leprince and Buitink 2010). DT during germination or post germination-DT is progressively lost until seedlings reach 'point of no return', after this point, seedlings no longer survive desiccation. Study of DT mechanism is very important in plant biology

Correspondence to: Vikramjit Kaur Zhawar
E-mail:vikram97jit@pau.edu

as this knowledge can be applied in many fields like crop breeding programs for improving stress tolerance or productivity, to build DT in recalcitrant seeds of economically important crops, somatic embryos/artificial seeds for their long-time storage (Nedeva and Nikolova 1997, Dekkers et al. 2015) and many non-plant applications.

Mechanism involved in post-germination DT is not known but ABA is the main regulator of the response (Maia et al. 2014). ABA improvement of post germination-DT is found in many plant species (Vieira et al. 2010, Maia et al. 2014, Masetto et al. 2014). ABA-insensitive mutants are found impaired in post germination-DT (Maia et al. 2014). PEG improvement of this DT is found (Vieira et al. 2010, Maia et al. 2011, Gaff and Oliver 2013, Masetto et al. 2014, Dekkers et al. 2015).

This study was aimed to study post-germination DT and its improvement by ABA or abiotic stress in wheat. ABA-pathways of vegetative stress response are different from pathways of its seed-responsiveness (Fujii et al. 2007). ABA-pathways of vegetative stress responsiveness belong to ROS/NO-pathways where ABA produces ROS or NO as mediators for its effects (Miller et al. 2010). These pathways provide tolerance to different abiotic stresses by reducing oxidative toxicity, increasing antioxidant activity and water retention (Miller et al. 2010). In the present study, ABA/ROS/NO-pathways were studied by using ROS/NO scavengers in control seedlings and ABA-treated seedlings.

MATERIALS AND METHODS

PLANT MATERIAL

Fresh seeds (freshly harvested stored at -20ºC) of wheat (*Triticum aesitvum*) cultivars PBW 644 and PBW 343 were seeded on sterilized filter paper moistened with autoclaved distilled water in petri-dishes, kept at 4ºC for 24 h to break dormancy, grown at 25ºC for 1 day, then, pre-treated with different treatments for 24 h, desiccated in desiccation chamber in the presence of saturated solution of $MgCl_2$ as desiccant for 4 d then rehydrated by transferring to autoclaved sand moistened with autoclaved distilled water for next 4 d (Maia et al. 2011). Different treatments included 20 µM ABA, 70% PEG 6000 as osmotic stress (OS), 300 mM NaCl as salt stress (SS), 40ºC as heat stress (HS), and distilled water as control (CT). Treatments for pathways included DMTU, tiron, PTIO, ABA plus DMTU/tiron/ PTIO where concentrations were 10 mM DMTU, 10 mM tiron, 50 µM PTIO. DMTU is N, N'- dimethylthiourea which is specific scavenger of H_2O_2. Tiron is sodium 4, 5-dihydroxybenzene-1, 3-disulfonate which is specific scavenger for superoxide anion radicals. PTIO is 2-phenyl-4, 4, 5, 5-tetramethylimidazoline-1-oxyl 3-oxide which is specific scavenger for nitric oxide. During the experiment, 3 stages were taken 2 d (germinated and treated), 6 d (desiccated), 10 d (rehydrated) old seedlings.

MEASUREMENT OF BIOMASS AND WATER CONTENT (WC)

Dry biomass of 50 seedlings was measured where fresh seedlings were dried at 80ºC for 16 h. Water content was calculated using following equation,

$$\mathrm{WC}\left(\text{g per g dw}\right) = \frac{\text{fresh biomass} - \text{dry biomass}}{\text{dry biomass}}$$

BIOCHEMICAL MEASUREMENTS

These were done on 25-50 seedlings. Protein content was extracted in 50 mM potassium phosphate buffer pH (7.0), 1 mM EDTA, 2% PVP and 0.05% triton-x-100. Protein content was measured by Lowry method. Nitric oxide (NO) (Kaur and Zhawar 2016) was extracted in 50 mM sodium acetate buffer (pH 3.6) with 4% zinc acetate and estimated by reacting 1 ml of appropriately diluted extract to 1ml of 1% sulphanilamide (in 5% phosphoric acid) and 1ml of 0.1% α-naphthylamine

(in ethanol) at 25ºC for 20 min. Absorbance was taken 530 nm and calculated using standard curve of nitrite (10-50 nmole). Protein carbonyls (Prasad 1996) were extracted in 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM PMSF and 0.5 µg ml^1 of aprotinin. Appropriately diluted extract (0.1 ml) was reacted to 0.5 ml of 10 mM 2, 4-dinitrophenyl hydrazine (prepared in 2N HCl) or 2N HCl (as control) for 1 hr in dark. Proteins were precipitated by adding 0.6 ml of 10% TCA on ice for 10 min and then centrifuged for 20 min to get the pellets. Pellets were dissolved in 6 M guanidine hydrochloride (pH 2.3) and absorbance was determined at 360 nm against blank. Carbonyl contents were calculated using $€_{hydrazone}$ 22, 000 M^{-1} cm^{-1}. Total protein content was estimated in same samples using Lowry method and carbonyl content was expressed as nmole mg^{-1} protein.

STATISTICAL ANALYSIS

Three biological replicates were taken for each measurement. Mean ± S. D. was calculated. Results were analyzed by Duncan Multiple test using DSAASTAT software.

RESULTS

All measurements were calculated per seedling basis as dry biomass, water content, protein content (Figs. 1, 3, 4) varied by significant amounts during the experiment. Data was observed in during desiccation (2 d to 6 d) and rehydration (6 d to 10 d) in CT seedlings of both cultivars for CT seedlings. Pretreatments were compared to CT and to one another at each stage, 2 d (before desiccation), 6 d (desiccated) and 10 d (rehydrated). ABA plus treatments (ABA+tiron/DMTU/PTIO) were compared with ABA to know the contribution of ROS/NO-pathways under ABA; tiron/DMTU/PTIO were compared with CT to know the contribution of ROS/NO-pathways in control seedlings.

BIOMASS

Biomass (Fig. 1) was increased from 2 d to 6 d (during desiccation) and 6 d to 10 d (during rehydration) in PBW 644 but in PBW 343, biomass was not increased from 2 d to 6 d but increased from 6 d to 10 d stages. ABA/stresses increased biomass at 6 d but decreased biomass at 10 d from their respective CT values in PBW 644, while in

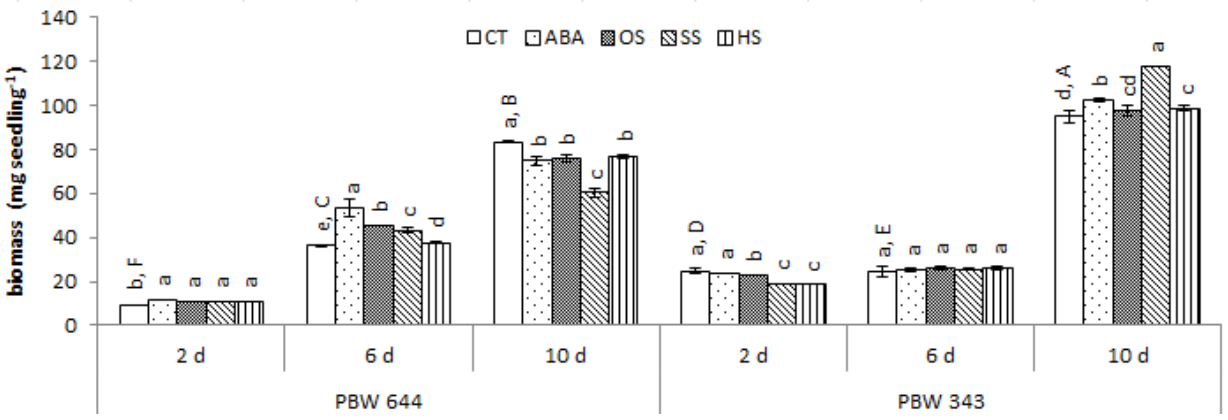

Figure 1 - Change of biomass during post-germination desiccation and subsequent rehydration in wheat cultivars PBW 644 and PBW 343 where 1 d old seedlings were exposed to ABA, osmotic stress (OS), salt stress (SS), heat stress (HS) for next 1 d, then desiccated for 4 d subsequently rehydrated for next 4 d. Data was taken at 2 d (before desiccation), 6 d (desiccated) and 10 d (rehydrated) stages. Different uppercase alphabets represent significant difference among all six CT values (three stages of both cultivars) and different lowercase alphabets represent significant difference among treatments and CT at each stage (Duncan Multiple test $p \leq 0.05$).

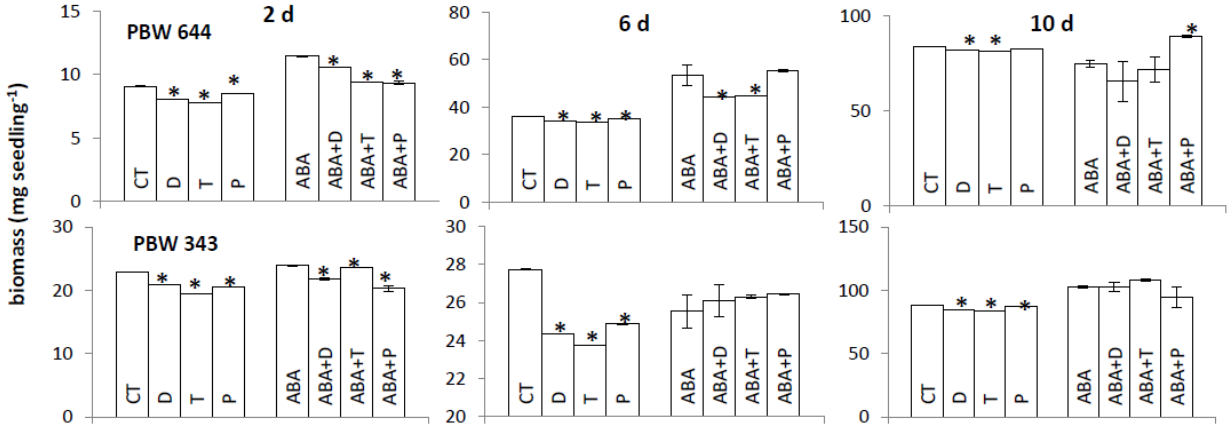

Figure 2 - ROS/NO regulation of biomass in CT and ABA treated seedlings of PBW 644 (upper panel), PBW 343 (lower panel) at 2 d (before desiccation), 6 d (desiccated) and 10 d (rehydrated) seedlings where 1 d old seedlings exposed to DMTU (D), tiron (T), PTIO (P), ABA, ABA plus D/T/P, water (CT) for next 1 d, then, desiccated for 4 d and subsequently rehydrated for 4 d. * represents significant difference from CT or ABA (Duncan Multiple test $p \leq 0.05$).

PBW 343, ABA/stresses did not increase biomass at 6 d but increased biomass at 10 d.

Removal of ROS/NO under CT (Fig. 2) reduced biomass at all three stages in both cultivars. Removal of ROS/NO under ABA affected biomass in both cultivars at 2 d and in PBW 644 only at 6 d stage.

PROTEIN CONTENT

Protein content (Fig. 3) was increased from 2 d to 6 d, from 6 d to 10 d in both cultivars but by higher amount in PBW 644. ABA/stresses increased protein content at 6 d and 10 d stages in both cultivars. Removal of ROS/NO under ABA decreased protein contents at 6 d and 10 d stages in both cultivars.

WATER CONTENT (WC)

WC (Fig. 4) was decreased during desiccation then, increased during rehydration, this decrease and increase was best shown in PBW 644 but not in PBW 343. WC of desiccated seedlings reached to value of 0.29 in PBW 644 but to 0.35 in PBW 343. In PBW 644, WC of desiccated seedlings was further decreased to the value of 0.13 by ABA and OS, to the value of 0.18 to 0.22 by SS and HS. In PBW 343, only OS decreased WC to the value of 0.26 while other stresses did not alter the WC.

In PBW 644, removal of ROS/NO under CT decreased WC (at 6 d and 10 d) but under ABA, removal of ROS/NO did not decrease WC (rather increase it) except at 10 d stage only, where removal of NO decreased WC. In PBW 343, only removal of NO under ABA decreased WC at 6 d stage.

OXIDATIVE TOXICITY (PROTEIN CARBONYLS)

PBW 343 showed very high increase in protein carbonyls (Fig. 5) under desiccation, this was not observed in PBW 644. ABA/stress pretreatments did not decrease protein carbonyls rather increased it by small amount under desiccation in both cultivars.

Removal of ROS/NO under CT did not increase protein carbonyls in both cultivars. But removal of ROS/NO under ABA increased protein carbonyls mainly in PBW 644.

NO

Both cultivars showed increase of NO (Fig. 6) during desiccation and rehydration. Rehydrated seedlings of PBW 343 showed comparatively higher level of NO compared to same seedlings of

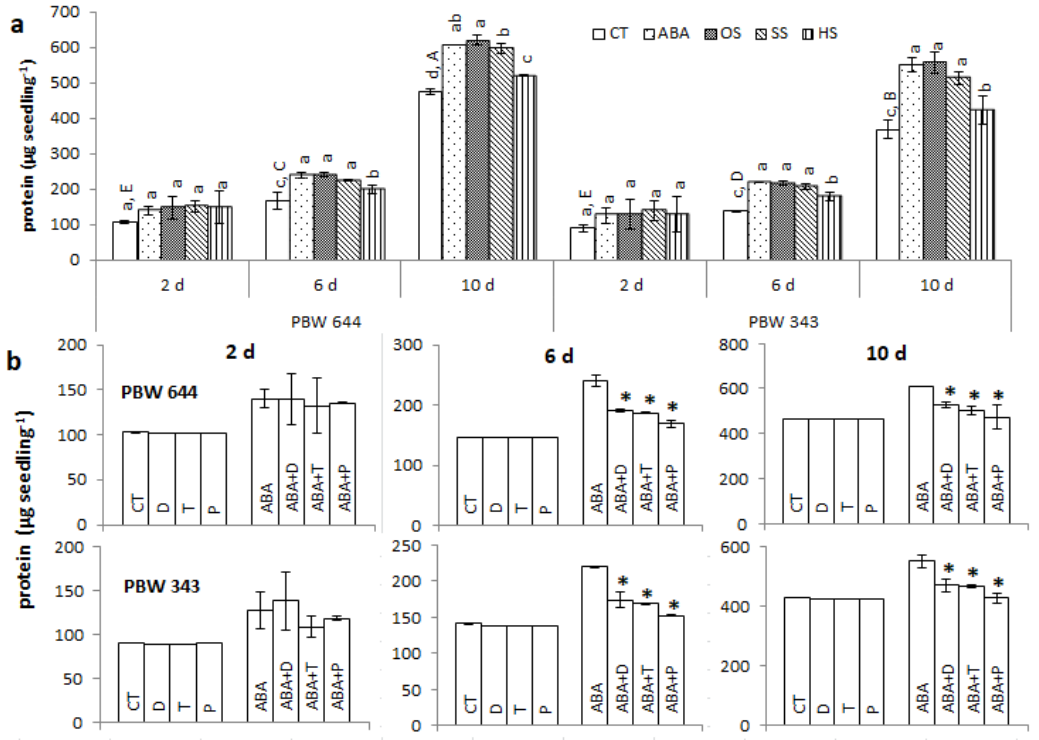

Figure 3 - Change of protein content (**a**) and ROS/NO regulation of protein (**b**) during post-germination desiccation and subsequent rehydration in wheat cultivars PBW 644 and PBW 343. Rest is same as in Fig. 1 for **a** and Fig. 2 for **b**.

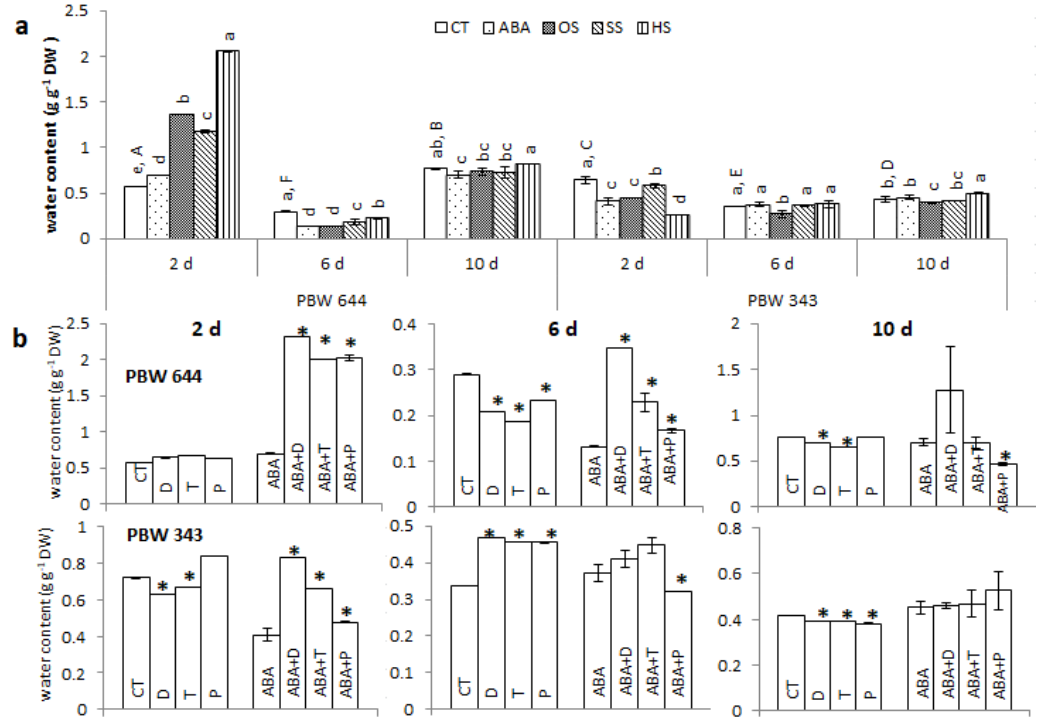

Figure 4 - Change of water content (WC) (**a**) and ROS/NO regulation of WC (**b**) during post-germination desiccation and rehydration of wheat cultivars PBW 644 and PBW 343. Rest is same as in Fig. 1 for **a** and Fig. 2 for **b**.

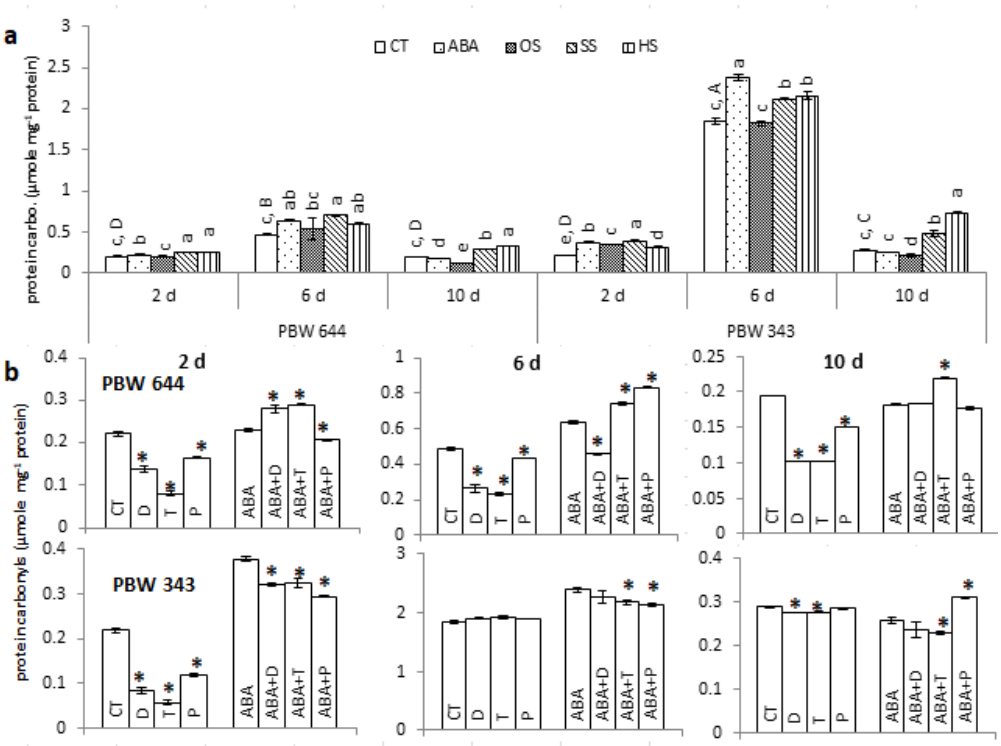

Figure 5 - Change of protein carbonyls (**a**) and ROS/NO regulation of protein carbonyls (**b**) during post-germination desiccation and rehydration of wheat cultivars PBW 644 and PBW 343. Rest is same as in Fig. 1 for **a** and Fig. 2 for **b**.

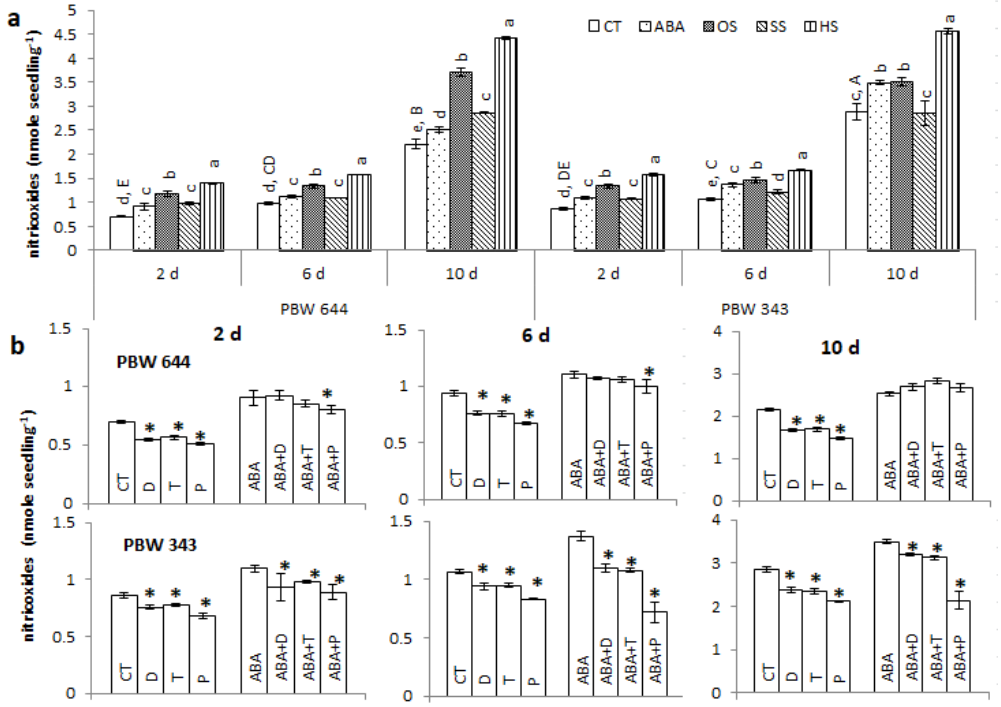

Figure 6 - Change of nitric oxide content (**a**) and ROS/NO regulation of nitric oxide (**b**) during post-germination desiccation and rehydration in wheat cultivars PBW 644 and PBW 343. Rest is same as in Fig. 1 for **a** and Fig. 2 for **b**.

PBW 644. ABA/stresses increased NO at almost all three stages in both cultivars. ABA increase of NO was higher in PBW 343 than PBW 644. Removal of ROS/NO under CT decreased NO at all three stages in both cultivars while removal of ROS/NO under ABA decreased NO mainly in PBW 343 at all three stages.

DISCUSSION

Biomass and water content measurement showed that PBW 644 increased biomass with loss of water during desiccation. This feature was ABA upregulated. Increase of biomass could be due to accumulations of high molecular weight compounds as protectants. This has been suggested (Buitink and Leprince 2008) that mechanism of desiccation involves replacement of cellular water molecules with high molecular weight compounds like LEA proteins, HSFs, non-reducing sugars sucrose, RFOs and antioxidants, these compounds make stable interactions with cellular components, form glassy matrix which prevents collapse of cellular structures, thus, helps in cell stabilization. ABA induces these protective molecules under desiccation (Vieira et al. 2010, Maia et al. 2011). ABA in its vegetative response is involved in water retention though its increasing effect on osmolyte accumulation (Miller et al. 2010) but ABA in seed desiccation studies is related to desiccation induced water loss as ABA-defective mutants produced seeds with high water content, remained green and desiccation sensitive (Nedeva and Nikolova 1997). ABA-treatment to artificial/somatic embryos reduced water level and induced DT, hence allow their preservations as synthetic seeds (Srinivas et al. 2006). Seeds of C 306 (ABA-higher sensitive, drought tolerant, higher dormant wheat cultivar) were found to contain lesser water content than of PBW 343 at both fresh and after-ripened states (Kaur and Zhawar 2016). By losing water during desiccation, PBW 644 might be using metabolic arrest for survival as it reduced ROS production (Huang and Song 2013). Decrease of biomass by ABA/stresses in rehydrated seedlings of PBW 644 also showed that desiccation was accompanied with higher growth arrest. On the other hand, PBW 343 did not increase biomass and did also not loose water effectively during desiccation, hence this cultivar might be having metabolic active state, therefore accompanied with higher oxidative toxicity. ABA/stress application did also not induce water loss but increased growth upon rehydration, this indicated that these treatments used growth promotive mode (drought tolerance) for survival. Nitric oxide measurements also showed that though both cultivars used endogenous NO-dependent tolerance in CT seedlings but in ABA-treated seedlings, PBW 343 was using more part of this tolerance. PBW 343 also used NO-signal to retain water during desiccation in ABA-treated seedlings.

Both cultivars used ABA/ROS/NO-pathways to maintain biomass and protein content during desiccation and subsequent rehydration where PBW 644 was using more effective such signaling. PBW 644 did not increase protein carbonyls during desiccation but did also not use ROS/NO-pathways to control it. Reason could be that it may either be using some other pathway to control it or toxicity may not be produced due to metabolically arrested state of its desiccation. However, under ABA, it used ROS/NO-pathways to control toxicity where PBW 343 was poor in it.

PBW 644 showed the involvement of ROS/NO-pathways in water retention in CT-seedlings but not under ABA/stress-treated seedlings. In *Arabidopsis thaliana* (Maia et al. 2011), CT-seedlings lost water rapidly to about 0.08 g per g dw by 6 hour and did not survive upon rehydration while PEG treated seedlings retained water till 0.5 g per g dw for first 72 hr of dehydration, then, decreased to 0.08 g per g dw, these seedlings showed 100% survival. In the present study, we did not get complete desiccation till 0.1 g per g dw

in any sample. Reason could be the different plant system. It is clear that water retention/water loss programme might be decided by some endogenous signal like amount of ABA, e.g., less intense stress signal may induce water retention but prolonged stress induces water loss programme. Different studies on DT like seed DT, DT of resurrections plants, post-germination DT (Nedeva and Nikolova 1997, Dinakar and Bartels 2013, Dekkers et al. 2015) have indicated a close similarity between DT and drought tolerance, ABA may also play similar roles, but significant differences exist, one such difference is water retention/water loss program, DT uses water loss to induce metabolic arrest while drought tolerance uses water retention to stay hydrated and metabolically active.

This study concluded that improved post germination-DT of PBW 644 could be due to ABA-regulated water loss program during desiccation, so to use metabolic arrest as survival strategy. On the other hand, PBW 343 appeared to be poor in water loss program, thus overproduced oxidative damage. Otherwise both cultivars used vegetative stress tolerant pathways of ABA/ROS/NO for improving DT, where PBW 644 showed more effective use of such pathways. Secondly, ABA-regulation of post-germination DT can be the mechanism of cross-tolerance as similarly observed under OS/SS/HS. Omics studies (Collett et al. 2004, Wang et al. 2012) have shown that DT could be the mechanism of cross-tolerance, even biotic stress related genes were found upregulated under DT. PBW 644 is more drought tolerant than PBW 343, so it may use DT during drought. In one such study on wheat cultivars (Lascano et al. 2001) during drought under field as well as *in vitro* conditions, lower WC has been related with drought tolerance and this ability of plant to maintain cell functions under low WCs has been suggested one of the mechanisms of dehydration tolerance (Lascano et al. 2001). Therefore, it could be that during vegetative growth of plant, some genotypes of the plants may be adapted to use DT under drought or other environmental stresses. Due to which, these genotypes may perform better under stresses.

REFERENCES

BUITINK J AND LEPRINCE O. 2008. Intracellular glasses and seed survival in the dry state. C R Biol 331: 788-795.

COLLETT H, SHEN A, GARDNER M, FARRANT JM, DENBY KJ AND ILLING N. 2004. Towards transcript profiling of desiccation tolerance in *Xerophyta humilis*: Construction of a normalized 11 k *X. humilis* cDNA set and microarray expression analysis of 424 cDNAs in response to dehydration. Physiol Plant 122: 39-53.

DEKKERS BJW, COSTA MCD, MAIA J, BENTSINK L, LIGTERINK W AND HILHORST HWM. 2015. Acquisition and loss of desiccation tolerance in seeds: from experimental model to biological relevance. Planta 241: 563-577.

DINAKAR C AND BARTELS D. 2013. Desiccation tolerance in resurrection plants: new insights from transcriptome, proteome, and metabolome analysis. Front Plant Sci 4: e482.

FUJII H, VERSLUES PE AND ZHU JK. 2007. Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in *Arabidopsis*. Plant Cell 19: 485-494.

GAFF DF AND OLIVER M. 2013. The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon. Funct Plant Biol 40: 315-328.

HUANG H AND SONG S. 2013. Change in desiccation tolerance of maize embryos during development and germination at different water potential PEG-6000 in relation to oxidative process. Plant Physiol Biochem 68: 61-70.

KAUR KK AND ZHAWAR VK. 2016. Antioxidant potential of fresh and after-ripened dry embryos of two wheat cultivars contrasting in drought tolerance. Indian J Plant Physiol 21: 350-354.

LASCANO HR, ANTONICELLI GE, LUNA CM, MELCHIORRE MN, GOMEZ LD, RACCA RW, TRIPPI VS AND CASANO LM. 2001. Antioxidant system response of different wheat cultivars under drought: field and *in vitro* studies. Aust J Plant Physiol 28: 1095-1102.

LEPRINCE O AND BUITINK J. 2010. Introduction to desiccation biology: from old borders to new frontiers. Planta 242: 369-378.

LYALL R, INGLE RA AND ILLING N. 2014. The window of desiccation tolerance shown by early stage germination seedlings remains open in the resurrection plant, *Xerophyta viscosa*. PLoS ONE 9: e93093.

MAIA J, DEKKERS BJ, DOLLE MJ, LIGTERINK W AND HILHORST HW. 2014. Abscisic acid (ABA) sensitivity

regulates desiccation tolerance in germinated *Arabidopsis* seeds. New Phytol 203: 81-93.

MAIA J, DEKKERS BJW, PROVART NJ, LIGTERINK W AND HILHORST HWM. 2011. The re-establishment of desiccation tolerance in germinated *Arabidopsis thaliana* seeds and its associated transcriptome. PLoS ONE 6: e29123.

MASETTO TE, FARIA JM AND FRAIZ ACR. 2014. Re-induction of desiccation tolerance after germination of *Cedrela fissilis* Vell. seeds. An Acad Bras Cienc 86: 1273-1285.

MILLER G, SUZUKI N, SULTAN CY AND MITTLER R. 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stress. Plant Cell Environ 33: 453-467.

NEDEVA D AND NIKOLOVA A. 1997. Desiccation tolerance in developing seeds. Bulg J Plant Physiol *23*: 100-113.

PRASAD TK. 1996. Mechanisms of chilling-induced oxidative stress injury and tolerance in developing maize seedlings: Changes in antioxidant system, oxidation of proteins and lipids, and protease activities. Plant J 10: 1017-1026.

SRINIVAS L, GANAPATHI TR, SUPRASANNA P AND BAPAT VA. 2006. Desiccation and ABA treatment improves conversion of somatic embryos to plant-lets in banana (*Musa* spp.) cv. Rasthali (AAB). Indian J Biotechnol 5: 521-526.

VIEIRA CV, AMARAL DA SILVA EA, DE ALVARENGA AA, DE CASTRO EM AND TOOROP PE. 2010. Stress-associated factors increase after desiccation of germinated seeds of *Tabebuia impetiginosa* Mart. Plant Growth Regul 62: 257-263.

WANG WQ, MOLLER IM AND SONG SQ. 2012. Proteomic analysis of embryonic axis of *Pisum sativum* seeds during germination and identification of proteins associated with loss of desiccation tolerance. J Proteomics 77: 68-86.