Integrating Transcriptome and Target Metabolome Variability in Doubled Haploids of *Allium cepa* for Abiotic Stress Prospecting

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Abstract Environmental stress conditions such as drought, heat, salinity, or pathogen infection can have a devastating impact on plant growth and yield, resulting in a mandate for stress-tolerant crop varieties. Crossbreeding tropical and cultivated onion species provided a hybrid F_1 generation possessing genetic and metabolic parental properties that aided abiotic stress tolerance. The targeted metabolite profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS) integrated with transcriptional analysis of their relevant genes, provide insight into the metabolic and genomic architecture between onion double haploid (*A. cepa* L., DHC), shallot double haploid (*A. cepa* L. Aggregatum group, DHA), and F_1 hybrid. Of a complete set of 113 targeted metabolites, 49 metabolites were found to be statistically different within genotypes; 11 metabolites were characteristic for DHC, 10 for DHA, 14 for F_1, and 14 metabolites were mutual among the three genotypes. Several key genes and metabolites introgressed in abiotic stress response have been up-regulated in DHA and F_1 genotypes as compared to DHC. Principal component analysis (PCA) and Volcano plot analysis revealed that metabolic traits and their relevant genes (*i.e*., amino acid, carbohydrate, flavonoid, and phospholipid biosynthesis) were strongly linked with DHA and F_1, reflecting the adaptability of DHA and F_1 toward abiotic stress as compared to DHC.

Keywords *Allium* · RNAseq · Metabolomics · Transcriptomics · Abiotic stress

Introduction

Onions ($2n$=16) are the third most important crop worldwide; most commercially grown cultivars are F_1 hybrids (Alan et al. 2003). Onions are generally considered to be drought sensitive; this limits its production to areas with adequate irrigation facilities, which influences the production price (Brouwer et al. 1989). Abiotic stresses have a significant economic impact; it has been suggested that they reduce the average yield by > 50% for most major crop plants (Atkinson and Urwin 2012). This is expected to increase drastically with global climate change, affecting global agricultural systems and resulting in a mandate for stress-tolerant crop varieties (Takeda and Matsuoka 2008; Newton et al. 2011). In Southeast Asian countries, shallots ($2n$=16) which are closely related to onions, are an economically important crop because of their pungency and adaptability to environmental stresses (Sulistyaningsih et al. 1997; Currah 2002); however, the molecular and metabolic architecture underlying this tolerability is still unclear. Inheritance of genetic materials from shallot to the Japanese bunching onion (*A. fistulosum*) enhanced *A. fistulosum's* flavonoid profile, one major metabolites that interacts with abiotic stress (Masuzaki et al. 2006), amino acid content and cysteine sulfoxide production during the summer season (Masamura et al. 2011), sucrose and fructan content in the winter season (Yaguchi et al. 2008), and shallot specific saponin compounds for fusarium basal rot resistance (Hoa et al. 2012). These reports gave insight into the potentiality of shallot as a prospective genetic resource for the future breeding of onions toward biotic and abiotic stress tolerance.

The shallot is a heterozygous species; therefore, hybrids of the shallot and the onion will show variations in many characters. This will hamper the progress of the breeding program. In addition, development of an inbred onion line may require 5-10 years, depending on the complexity of the traits under selection. Double haploid (DH) techniques that use the chromosomal doubling of haploid plant (n=8) can shorten the time needed, offer homozygous pure lines, and provide precious materials for genomic analysis (Sulistyaningsih et al. 1997; Alan et al. 2003). With the development of genomic initiatives to outline genome and gene expression in the context of plant-environment interaction, metabolic profiling is required for a better understanding of plant response mechanisms against various environmental stresses (Peremarti et al. 2014). MS-based metabolomics allow concomitant detection of several hundred metabolites as a snapshot of metabolomics phenomena (Sawada et al. 2009). Integrated metabolomics, genomics, and transcriptomics, referred to as *omics technologies*, play an important role in phytochemical genomic and crop breeding in sequenced plants (Saito and Matsuda 2009).

In this context, knowledge of the environmental stress adaptability of DHA, a cultivar of tropical origin, as compared to DHC is gaining importance. The aim of the present study was to discriminate between DHC, DHA, and F_1 genotypes using target metabolite analyses through chemical assignment for major metabolic classes, including metabolism of amino acids, carbohydrates, phospholipids, flavonoids, and alkaloids by LC-MS/MS integrated with the transcriptional profiling of their relevant genes under normal conditions. The obtained data will be useful for elucidating gene function for understanding biological variations in DHC, DHA, and F_1 genotypes and for demonstrating a technical ability to reveal the expected coherence between metabolic traits and abiotic stress parameters in the context of *Allium* genotypes.

Materials and Methods

DHC, DHA, and F_1 production

Shallot strain 'Chiang mai' from Thailand and the long-day onion stain 'Sapporo-ki' from Japan were used to establish the DHC (*A. cepa* L., DHC) and DHA (*A. cepa* L. Aggregatum group, DHA) according to Sulistyaningsih et al. (2006), with some modifications (Fig. 1a, b). DHC grown in pots were used as donors and DHA was used as recipients for crossing to obtain F_1 hybrids. The three genotypes were grown under the same conditions in the glasshouse at Yamaguchi University, Faculty of Agriculture using earthen pots filled with sandy soil. The average temperature was 18 ± 2°C, relative humidity 78% and 10 h daylight length. Water and fertilizers were applied equally for each genotype on weekly base. Plants were grown in randomize block design, each genotypes were grown in a single pots with total nine pots for the three genotypes. The whole leaves from 2-3 biological replica of each genotypes were harvested on the middle of November and freeze dried using VD-250R Taitech. The freeze dried samples from each genotypes were powdered and divided into three analytical replica for metabolic extraction.

Total RNA extraction and transcriptional analysis

The total RNA of the DHC, DHA, and F_1 was isolated from the fresh leaf tissue using RNeasy Plant Mini Kit (Qiagen, Tokyo, Japan). Using the total RNA of each plant, cDNA was synthesized using a First Strand cDNA Synthesis kit (Roche Diagnostics, Mannheim, Germany) with AMV reverse transcriptase. Next-generation sequencing for the isolated RNA was carried out at the Bejin Genomic Institution (BGI) using the Illumina GA/HiSeq System (HiSeq 2000 series) combined with HiSeq control software for program control, real-time analyzer software to do on-

instrument base-calling, and CASAVA for secondary analysis. The gene expression dataset was uploaded in the Allium Transcript Database (AlliumTDB, http://allium.kazusa.or.jp/).

Extraction of Metabolites

The leaf tissue samples of each genotypes were pooled, freeze-dried, powdered, and divided into three group representing three analytical replication. Freeze-dried samples (4 mg D.W.) in 2-mL tubes were extracted with 80% MeOH that contained 2.5 μM lidocaine per mg of dry weight using a mixer mill (Shake Master Neo, BMS, Tokyo, Japan) with 5 mm zirconia beads for 1000 rpm for 5 min at 4 °C. After centrifugation for 10 min, the liquid samples were prepared using a liquid handling system (MICROLAB STAR PLUS, Hamilton Co., Reno, NV, USA); 50 uL of the supernatant was transferred to a 96-well formatted collection plate and they were dried, resulting in 250 uL of LC-MS grade pure water.

LC−MS analysis

A liquid sample (1 μL) was analyzed using the UPLC-TQS (Waters Corp., Watertwon, MA, USA). Analytical conditions were as follows. LC: column, ACQUITY UPLC HSS T3 (1.8 μm, 1.0 mm ×100 mm, Waters Corp.); solvent system, solvent A (water with 0.1%formic acid) and solvent B (acetonitrile with 0.1% formic acid); gradient program, 99.9% A/0.1% B at 0 min, 99.9% A/0.1% B at 0.25 min, 91.0% A/0.9% B at 0.40 min, 83.0% A/17.0% B at 0.80 min, and 0.1% A/99.9% B at 1.90 min, 0.1% A/99.9% B at 2.10 min; 99.9% A/0.1% B at 2.11 min flow rate, 0.240 mL/min.

Results

Metabolic variations in DHC, DHA, and F_1

A total of 113 distinct metabolites were positively annotated using reference data of the retention time and the mass spectrum with authentic compounds. To validate the biological data based on a statistically significant difference, PCA loading plots were performed on the metabolite dataset (113 metabolites, 3 genotypes, 3 leaf tissues) to build models that can visualize the relationship between the metabolite signature and the genotypes (Fig. 2). Clear discrimination of the three genotypes showed 85.8% of the variance in PC1 and 7.2% in PC2 with an accumulative proportion of variance of 93%. The entire dataset was further analyzed using a Volcano plot (Fig. 3a, b, c) to give a scatter overview of the metabolites significance (y-axis) versus magnitude of the change (x-axis) between the three genotypes. (DHA vs. DHC, F_1 vs. DHA, and F_1 vs. DHC). The metabolites with low p-values (highly significant) appearing toward the top of the

plot however, the log of the fold-change is used so that changes in both directions (right and left) appear equidistant from the center. Of the complete set of 113 metabolites, 49 metabolites were found to be statistically different within the genotypes (Log10 $P< 0.05$ and log2 fold changes > 1.2). The genotype-metabolite fingerprints are demonstrated in supplementary data (S1). To determine the metabolite flux, a Venn diagram of the compounds whose accumulations were significantly ($P> 0.05$) altered by genotype carried out. Of the 49 metabolites, 10 metabolites were characteristic for DHC, 11 for DHA, and 14 for F_1, and 14 were mutual among the three genotypes. It was interesting to observe that of the 14 mutual metabolites, 10 were shared between DHA and F_1, three between DHC and F_1, and one between DHC and DHA. This observation indicates that the metabolic flux in the F_1 genotype was mainly directed from the DHA metabolic pool rather than that of DHC, which may offer a prediction as to the capability of F_1 as stress-tolerant candidate, emphasizing the potential of DHA as an important bioresource for plant breeding. The identified metabolites represent a small number of distinct metabolic classes, the most predominant involving amino acid, organic acid, and carbohydrate metabolism (including monosaccharaides, oligosaccharides, and sugar alcohol), shikimate pathway metabolism (including phenylpropanoids and alkaloids), and phospholipid metabolism. In the present study, DHA and F_1 showed a distinctive accumulation of free aromatic and non-aromatic amino acids and their derivatives in compare with DHC genotype. Organic acids and their derivatives were detected in DHC, including Aspartic acid and beta-homoglutamine, however, malic acid was specific to DHA and F_1. The most important metabolic variation was observed in the carbohydrate metabolism. An increase in carbohydrates was detected in the DHA and F_1, which seem to be a characteristic feature for those genotypes as compared to the DHC. Furthermore, an important signaling molecules and osmolytes that influenced the plant's stress response were exclusively detected in DHA and F_1 such as alkaloids (trigenolline and indole-3-carboxaldehyde), flavonoids (luteoline, kampferol, quercetin, and cyanidine glucoside), phospholipid (phosphocholine, glycerol-3-phosphate, glycerol-3-phosphocholine, and Glyceraldehyde-3-phosphate), quaternary ammonium compounds (carnitine and dimethylglycine), and vitamins including vitamin B6 (pyridoxine) and Vitamin B5 (pantothenic acid). DHC accumulated an important class of quaternary ammonium compounds such as betain and dimethylglycine, in addition to nucleoside molecules, such as inosine and cytidine.

Transcriptomic variations in DHC, DHA and F_1

Transcriptional profiling revealed differences in gene expression between the three genotypes under normal conditions. The gene expression dataset was uploaded in the *Allium* Transcript Database (*Allium*TDB, http://allium.kazusa.or.jp/).

We initially compared the gene expression of DHA and F_1 genotypes with the DHC as a control to identify genotype-specific stress-related genes. A total of 77 gene expression (log2 RPKM values) representing the following functional categories of primary and secondary metabolism was illustrated with heatmap2 using open source statistical package R, version 3.0.2 (http://www.r-project.org/). The amino acid biosynthesis genes showed up-regulation in the DHA and F_1 as compared to the DHC (Fig. 4a). This includes the genes that encode homoserine dehydrogenase (*HSD*), homoserine kinase (*HSK*), threonine synthase (*TS*), cystathionine-γ-synthase (*CGS*), methionine synthase (*MS*), threonine deaminase (*TDA*), *S*-adenosylmethionine synthase (*SAMS*), *S*-adnosylmethionine decarboxylase (*SAMDC*), threonine aldolase (*THA*), 1-aminocyclopropane-1-carboxylate synthase (*ACCS*) and 1-aminocyclopropane-1-carboxylase oxidase (*ACCO*) with 2.54-, 2.45-, 2.13-, 2.82-, 4.26-, 3.19-, 2.95-, 1.70-, 3.23-, 4.21-, and 2.22-fold increases, respectively in DHA and 1.43-, 1.09-, 1.88-, 2.50-, 1.62-, 5.24-, 2.37-, 3.14-, 6.22-, 3.15-, and 1.59-fold increases in F_1, respectively, as compared to DHC.

Similarly, transcriptomic profiling revealed differences between the genotypes in the regulation of carbohydrate metabolism (Fig. 4b). The synthesis of osmoprotectants such as ROFs was specifically up-regulated in the DHA and F_1, including the genes encoding galactokinase (*GALK*), α-galactosidase (*GAL*), galactinol synthase (*GolS*), and raffinose synthase (*RS*) with 2.16-, 2.04-, 4.70-, and 3.04-fold increases in DHA and 1.24-, 1.16-, 17.70-, and 10.98-fold increases in F_1, respectively. The DHA and F_1 genotypes appeared to promote sucrose and alcohol sugar synthesis, including those genes encoding sucrose synthase (*SucS*), Hexokinase (*HK*) and Mannose-6-phosphate isomerase with 4.92-, 2.14-, and 2.17-fold increases in DHA and 1.63-, 1.75-, and 7.25-fold increases in F_1, respectively. In addition, genes involved in diverting the carbon flux to monosaccharide and polysaccharide syntheses that encode cellulose synthase (*CS*), xylose isomerase (XylA), deoxyxylulose-5-phosphate synthase (*DXS*), UDP-glucose-4 epimerase (*GluE4*),UDP-glucose 6-dehydrogenase (*GlucDH*), and UDP-arabinose 4-epimerase (*AraE4*) were up-regulated in DHA (5.62-, 2.44-, 1.98-, 1.83-, 3.79-, and 4.0-fold changes, respectively) and F_1 (2.83-, 2.33-, 1.99-, 7.53-, 3.78- and 3.16-fold changes, respectively).

Shikimate, flavonoid and phospholipid metabolism were similarly up regulated in DHA and F1 (Fig. 4c). This includes genes that encode phosphoenolpyruvate carboxykinase (*PCK*), shikimate kinase (*SK*), 3-dehydroquinate synthase (*3DHQS*), 3-phosphoshikimate 1-carboxyvinyltransferase (*AroA*), and chorismate synthase (*AroC*) were highly expressed in DHA with 4.71-, 2.25-, 2.88-, 3.61-, and 2.88-fold changes and 2.61-, 1.89-, 1.77-, 2.10- and 3.07-

fold changes in F_1, respectively. These genes direct the carbon flow to chorismate synthesis, which is considered a branching point to the phenylpropanoid and tryptophan synthesis pathways. Phenyl-amonia lyase (*PAL*) and 4-coumarate CoA ligase (*4CL*), representing the upstream of the phenylpropanoid and flavonoid pathways were up-regulated in DHA (1.70- and 6.03-fold increases, respectively) and F_1 (1.61- and 5.10-fold increases, respectively). Flavonoid biosynthesis genes that encode flavanone 3-hydroxylase (*F3H*), dihydroflavonol 4-reductase (*DFR*) and leuco anthocyanidin dioxygenase (*LACDO*) were also highly expressed in DHA with 1.81-, 13.33-, and 31.30-fold increases, respectively, and in F1 with 5.99-, 11.22-, and 38.21-fold increases, respectively. Moreover, genes involved in the tryptophan and trigonelline synthesis that encodes anthranilate synthase (*ASA*), anthranilate phosphoribosyltransferase (*APRT*) and nicotinamidase (*PNC*) were up-regulated in DHA (3.17-, 1.85-, and 2.40-fold changes, respectively) and F_1 (2.0-, 1.99-, and 2.18-fold changes, respectively). The phospholipid metabolism that includes genes that encode phosphorylcholine cytidylyltransferase (*CCT*), phospholipase-A2 (*PLA*2), lysophospholipase (*LysoPL*), Glycerol phosphoryl diesterphosphodiesterase (*GP-PDE*) and glycerol-3-phosphate dehydrogenase (*GP-DH*) were up-regulated in DHA with 5.05-, 3.44-, 1.61-, 2.71-, and 2.15-fold increases, respectively, and in F_1 with 3.82-, 2.05-, 1.55-, 2.16-, and 2.58-fold increases respectively. These genes are responsible for directing the lipid pathways into glycerol-3-phosphocholine and glycerol-3-phosphate synthesis.

Discussion

To explore the genetic variation between DHC, DHA and F_1 genotypes, the association between targeted metabolic profiling and the transcriptomic level was investigated under normal conditions. At the transcriptional level, substantial variation has been observed among genotypes; many of these transcripts seem to be tangled in different aspects of primary as well as secondary metabolism. Amino acid, carbohydrate, alkaloid, phospholipid, and flavonoid metabolisms showed a significant variations ($P > 0.05$) among genotypes, suggesting that these metabolic changes may reflect genotype dependent and genetic adaption to environmental stimuli due to the plant origin as in the case of DHA which originated from tropical environment. Such trends in metabolic accumulation were reported in different plant stress tolerance (Parker et al. 2009; Evers et al. 2010; Dauwe et al. 2012)

The induction of free amino acids as osmolytes in response to abiotic stress is thought to play a pivotal role in plant stress tolerance through intracellular pH regulation, detoxification of reactive oxygen species, xenobiotics, UV, and heavy metals (Nuccio et al. 1999; Zagorchev et al. 2013). Threonine and methionine, substrates for isoleucine synthesis

via aspartate-derived pathway, were significantly higher in the DHA and F_1 genotypes as compared to the DHC genotype. *HSK* catalyzes the formation of *O*-phosphohomoerine from homoserine and leads to the formation of threonine or methionine through competitive affinities of the threonine synthase (*TS*) and *CGS* (Curien et al. 1996; Joshi et al. 2010). In the case of methionine catabolism, *SAMS* directs 80% of the metabolic flux of methionine into S-adenosylmethionine which is used to methylate nucleic acid, proteins and many plant metabolites (Joshi et al. 2010). Our results clearly show the overexpression of *TS*, *MS*, *CGS, TDA,* and *SAM* genes in DHA and F_1, which is in accordance with the same tendency of expression in stress-tolerant plants under drought and osmotic and salt stresses (Wu et al. 2004; Mitsuda and Ohme-Takagi 2009). Recent studies have suggested that threonine and methionine regulate isoleucine homeostasis under osmotic stress conditions; for example, increased isoleucine accumulation was observed in potatoes' *TS* expression (Zeh et al. 2001), potatoes *CGS* overexpression (Dancs et al. 2008), and *Arabidopsis CGS* expression (Hacham et al. 2008). Methionine is also a substrate for the synthesis of various important stress tolerance polyamines, such as putrescine, spermidine, and spermine (Alcázar et al. 2010), and this pathway involves S-Adenosylmethionine as a primary methyl donor. Moreover, S-adenosyl methionine is also a source for ethylene synthesis (van de Poel et al. 2013), reinforcing the pivotal role of methionine in plant stress responses. The overexpression of the threonine and methionine metabolism pathway in DHA and F_1 highlights the correlation between metabolic and genomic dataset and reflects the adaptability of DHA and F1 to abiotic stress as compared to DHC.

In addition to non-aromatic amino acids such as methionine, aromatic amino acids such as tryptophan were characteristic in DHA and F_1. Our results show the same tendency of gene expression as in *Arabidopsis* plants under oxidative stress by increasing the tryptophan biosynthesis pathway (*TrpA* and *TrpB*), suggesting that there is a coordinating regulation of the entire tryptophan pathway and stress responsive mechanism (Zhao et al. 1998).

Abiotic stress tolerance in plants, particularly drought tolerance, is intricately linked to carbohydrate metabolism (Basu et al. 1999). Sugars have been shown to modify transcription of many stress-related genes (Gupta and Kaur 2005). In the present frame work, DHA and F_1 showed a significant accumulation of the raffinose family oligosaccharides (RFOs), mannose-1, 6 phosphate,arabinose, and xylulose as compared to DHC, which might display a protective and adaptive role in DHA and F_1 genotypes under environmental stresses. Such a role has been proposed for *Pseudosuga menziesii* genotypes and environmental stress interaction, resulting in a distinctive accumulation of 19 metabolites that exhibited significant variation, among which raffinose, arabinose, xylose, malic acid, and alanine were

positively identified (Robinson et al. 2007). These forms of sugar are essentially entangled in the plant stress responses, acting as ROS scavengers, cell wall reinforcements, and osmoprotectants that stabilize cellular membranes and maintain turgor (Peshev and Van den Ende 2013; Keunen et al. 2013). ROFs are synthesized from sucrose, where *GolS* catalyzes the first step in the biosynthesis of RFOs, and *RS* catalyzes the synthesis of raffinose from galactinol (Lehle and Tanner 1973; Saravitz et al. 1987). Recently, it was reported that the expression of RFO biosynthesis genes is closely associated with the response to environmental stress. The transcriptional levels of *GolS* and *RS* increased under oxidative damage caused by drought, salt, or heat stress (Taji et al. 2002; Peter et al. 2007; Nishizawa et al. 2008). Interestingly, the F_1 genotype in the present study was characterized by high raffinose accumulation and up-regulation of *GolS* and *RS* as compared to DHC. The other important sugar class is sugar alcohols or polyols, which are often referred to compatible solutes; they function as osmoprotectants by forming an artificial sphere of hydration around the macromolecules, thus preventing metabolic inactivation under osmotic stress conditions (Williamson et al. 2002). Mannitol is a common six-carbon sugar alcohol synthesized from mannose-6-phosphates through the action of an NADPH-mannose-6-P reductase (*M6PR*) that catalyzes the conversion of mannose-6-P to mannitol-1-P (Rumpho et al. 1983). Mannitol is a potent quencher of ROS generated by abiotic stress (Williamson et al. 2002). The accumulation of mannose-1-P and mannose-6-P in DHA and F_1 would be an intermediate step in the mannitol downstream pathway, adding more insight of stress tolerance for DHA and F_1.

Arabinose and xylulose were specific for DHA and F_1. Arabinose is one of the main components of the pectin polymer side chain and a primary constituent of higher plant cell walls. Moore et al. (2006) reported that *Myrothamnus flabellifolia* leaves composed of an abundance of arabinose polymer side chains showed extreme flexibility in response to water loss and severe dehydration, suggesting a potential role of pectin-associated arabinose polymers in relation to water deficiency. Coffee plants subjected to heat stress (37 ºC) accumulated a higher content of arabinose in their leaf cell-walls, demonstrating the role of the composition cell-wall polymers during heat stress (Lima et al. 2013). Furthermore, Arabinose is a major component of Hyp-rich glycoproteins (HRGPs), which are believed to play a structural role in strengthening cell walls and are expressed in response to pathogen attack (Burget et al. 2003). Transcriptional analysis showed strong up-regulation of *GluE4* and *AraE4* in DHA and F_1, as these genes contribute to the regulation of the monosaccharide pool. The same finding was reported in potato leaves and transgenic *A. thaliana,* with an increase in *GluE4*-conferred tolerance to drought and salt stress (Evers et al. 2010; Liu et al. 2007).

Trigonelline the *N*-methyl conjugate of nicotinamide, is often classified as a pyridine alkaloid. It has proven to be a key metabolite that serves as a potent inducer of the defensive metabolism in plants, including the glutathione metabolism, and the accumulation of secondary defense compounds (Berglund 1994). Trigonelline may function as signal transmitter in response to oxidative stress caused by strong UV-B (Minorsky 2002). It is interesting to note that trigonelline was remarkably high in DHA and F_1 as compared to DHC. This finding is in accordance with previous reports regarding the accumulation of trigonelline in stress-resistant plant, which supports our hypothesis regarding DHA as a potential resource for stress tolerance. Alfalfa plants undergo a 2-fold increase in trigonelline after salt stress, and potato leaves under drought stress exhibited a 2.39-fold increase, proposing a potential role for trigenolline as an osmoregulator (Evers et al. 2010). Moreover, the metabolic dynamics under cold stress of *Picea sitchensis* populations revealed a 1.7 to 2.6-fold change in relation to the interaction of population and cold stress (Dauwe et al. 2011). The biosynthesis pathway of trigonelline that is involved in the conversion of nicotinamide into nicotinate and regulated by *PNC* was strongly up-regulated in the DHA and F_1 genotypes. The final step involved the methylation of nicotinate by nicotinate methyltransferase (*NMT*) using an S-adenosyl-methionine pool as a methyl donor, and thus limiting oxidative stress-induced DNA methylation (Wood 1999).

In the current study we observed an up-regulation of the phospholipid pathway (*CCT*, *LysoPL*, *GPDH,* and *GP-PDE*) and their corresponding metabolites in DHA and F_1. Glycerol-3-phosphate, a characteristic metabolite in DHA and F_1 is an important metabolite that serves as a precursor for the biosynthesis of all plant glycerolipids that contribute to growth and disease-related physiologies (Ghanda et al. 2011). Shen et al. (2006) reported the potential involvement of the glycerol-3-phosphate shuttle in plant redox control that contributes to maintaining the metabolite pools in a relatively constant state under different growth and stress conditions.

The metabolic and transcriptic dataset can be clearly linked with abiotic stress factors, as was undertaken in the present study, to establish a revealed picture of the differing metabolisms of onion and shallots that will facilitate our understanding of the biological process in these genotypes and enhance the onion-breeding program’s efforts toward a stress-tolerant varieties.

Conclusion

A combination of a broad-scale metabolic and transcript dataset provided insight into the molecular architecture of adaptive variation in DHA and F_1 of tropical origin as compared to DHC genotypes under normal conditions. The

increase of certain metabolites and their relevant genes expressed in the DHA and F_1 genotypes gave a better understanding of the molecular genetic determinants of adaptive trait variation with DHA' potential as an important resource for an onion- breeding program geared towards environmental stress tolerance.

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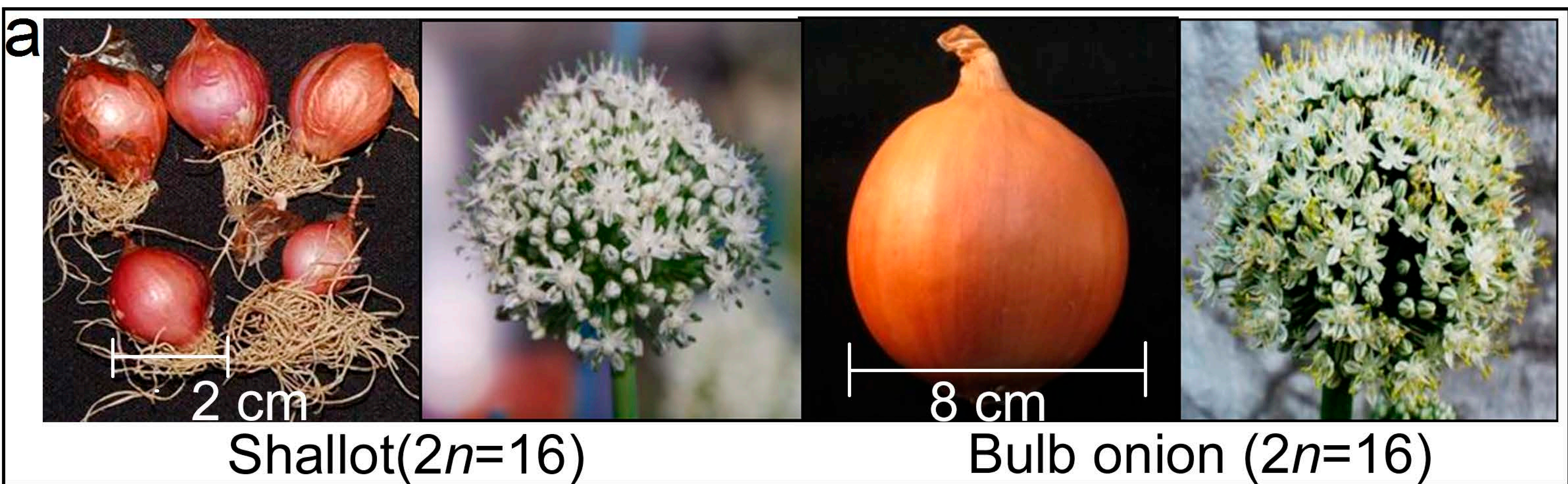
Footnote

Fig. 1 Plant materials production: long-day bulb onion “Sapporo-ki” and shallot “Chaing-mai” (a) and Scheme diagram for DHC, DHA and F1 production (b)

Fig. 2 PCA loading plots of LC-MS/MS data representing the metabolites discrimination among the three genotypes (DHC, DHA and F_1) with percentage of variance captured by each PC

Fig. 3 Volcano plots of genotype-metabolic comparison: DHA versus DHC (a), F_1 versus DHA (b) and F_1 versus DHC (c). Significant difference with $P< 0.05$ and >1.2-fold intensity ratio are shown as black spots. Gray spots mean no significant differences

Fig. 4 Model summarizing metabolites change and gene expression analysis associated with amino acid biosynthesis pathway (a), carbohydrate biosynthesis pathway (b) and shikimate, flavonoid, tryptophan, and phospholipid biosynthesis pathway (c) in the DHC, DHA and F_1. Metabolites and genes with significant accumulation ($P< 0.05$) in DHA and F_1 highlighted with red color and DHC with blue



b

Shallot
(South-East Asia ‘Chiang mai’)

Bulb onion
(Long-day ‘Sapporo-ki’)

Unpollinated flower culture

Shallot haploid
(*n*=8)

Bulb onion haploid
(*n*=8)

Chromosome doubling

Shallot doubled haploid(♀)
DHA

X

Bulb onion doubled haploid(♂)
DHC

F_1 hybrid

Fig. 1

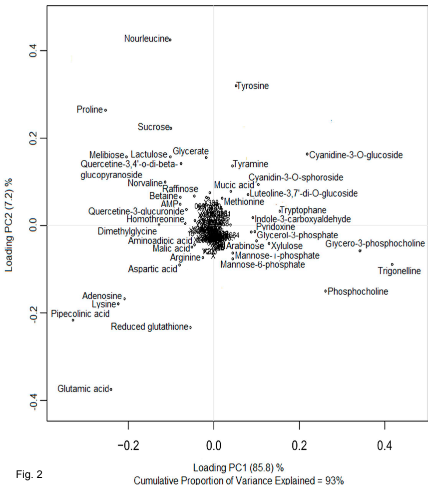


Fig. 2

Fig. 3

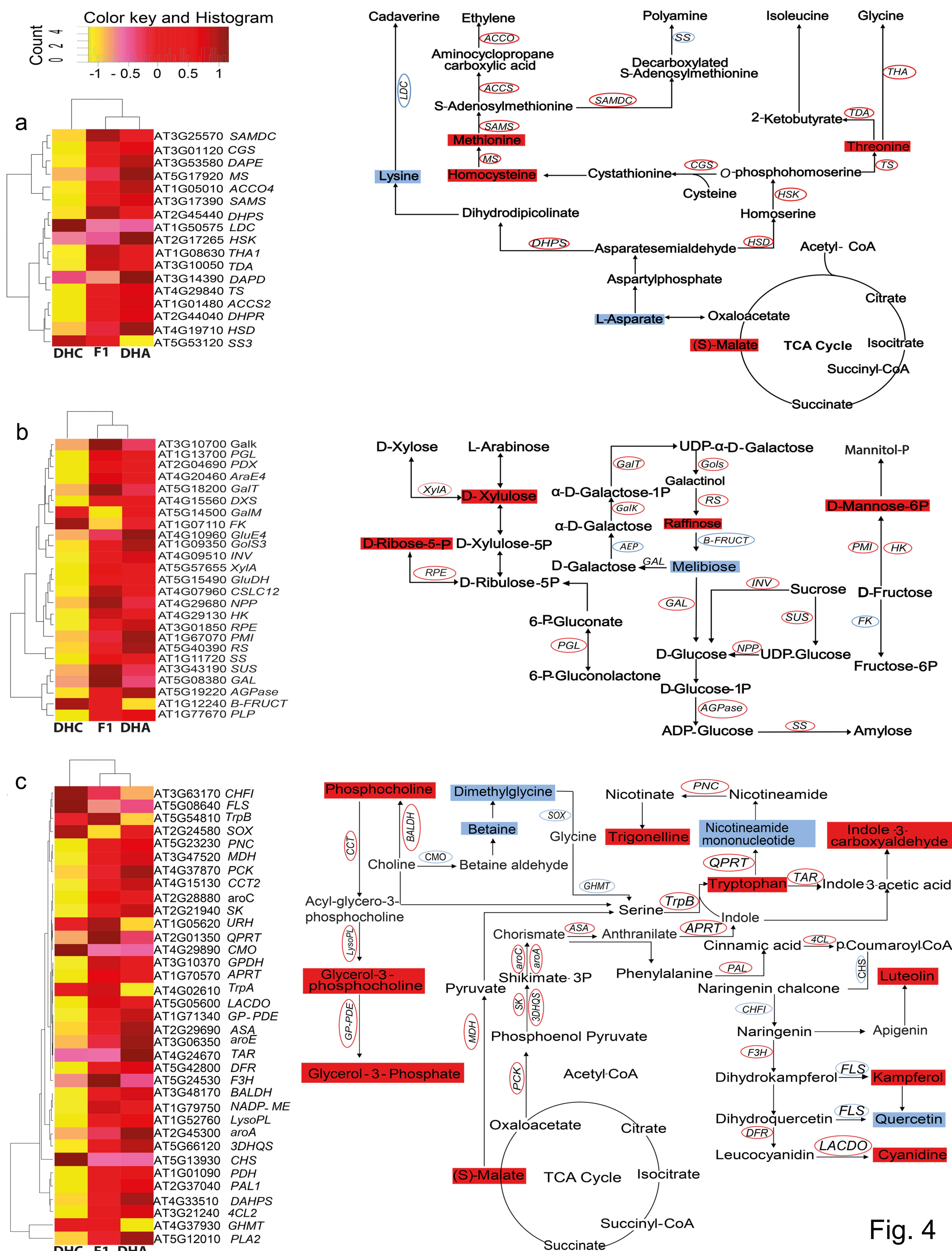


Fig. 4