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Brief Report

Diversity Control in Early Transcribed ROS/RNS-balancing Genes: A Common Mechanism for Healthy Resilience?

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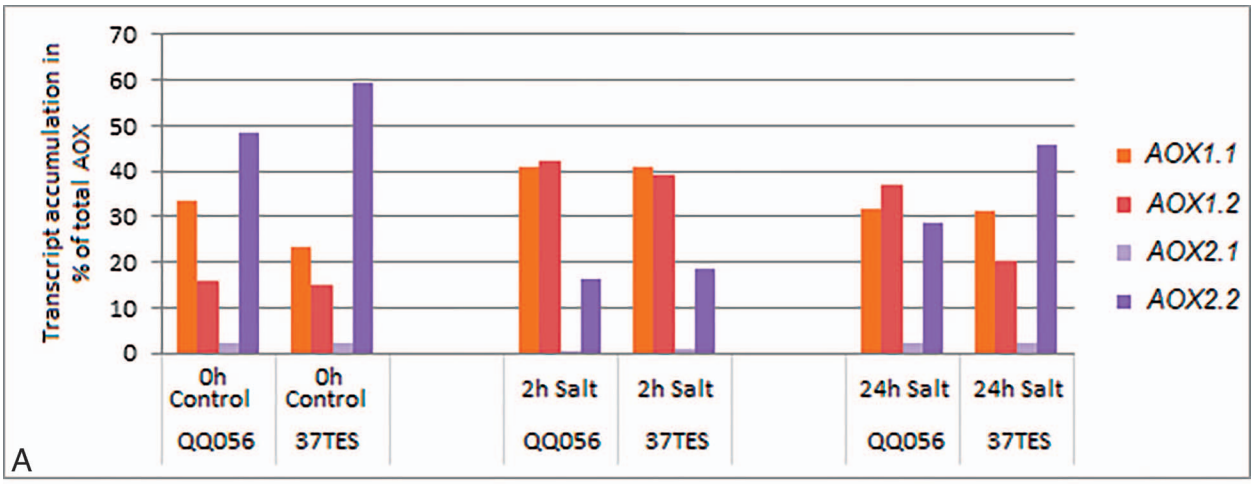
Recently, we highlighted adaptive reactive oxygen species (ROS)/reactive nitrogen species (RNS) equilibration in severe acute respiratory syndrome coronavirus 2–(SARS-CoV-2-) and influenza H3N2–infected human nasal epithelial cells (NECs) and in diverse plant systems as part of a major complex trait that marks stress-induced early cell reprogramming.[1–3] We hypothesized that sequence diversity in the plant gene alternative oxidase (*AOX*) and in the human N-acetylserotonin methyltransferase-like gene (*ASMTL*) could be an important trait per se for genotype-dependent and individual resilience prediction. Both genes were identified as reasonable markers for early ROS/RNS balancing.[4,5] Here, we report about essential new insights from novel data for driving this hypothesis.

It is current knowledge that ROS are important early indicators for all types of abiotic and biotic stresses that emerge in the primary target cells under ever-changing environmental conditions, including high salt in soils and virus attacks.[1,3,6] Quinoa is a halophyte plant that had been considered by the United Nations Food and Agricultural Organization as a relevant crop to ensure food security at global scale; 2013 was declared the International Year of Quinoa. This worship is because of the high nutritional quality of quinoa and its resilient field performance under a wide range of stress conditions. However, the secrets of why quinoa is acclimating so well to a diversity of stress factors remains obscure, but ROS scavenging was suggested to be a crucial factor under salt stress.[6]

Validation of the association of genes to resilient performance is easier in plants than in humans through top-down human-defined traits.[4] For our study, first we newly identified four *AOX* genes in the quinoa genome and the complementary DNAs were deposited in the Refseq-RNA database (accession no. XM_021912663.1, XM_021869875.1, XM_021920640.1, and XM_021897503.1). We verified that in this species, which evolved as an allotetraploid hybrid with subgenomes from two parental *Chenopodium* species, AOX is encoded by a small *AOX* gene family that involves two *AOX1* and two *AOX2* genes. Both genes in each AOX subfamily demonstrated approximately 96% identity. Next, we confirmed from public transcriptomic experimental data (Bioproject PRJNA636120, National Center for Biotechnology Information) that total AOX showed rapidly high levels of transcript accumulation in two varieties of quinoa when a salt stress was set (Fig. 1A). High initial *AOX* transcript level followed by efficient downregulation discriminated salt tolerant from susceptible rice varieties.[4,7] Such early *AOX* expression profile changes measured directly at the level of transcription or indirectly at the level of AOX activity by using an AOX inhibitor was recently highlighted as promising complex trait for general resilience prediction from seeds.[8,9]

In Figure 1A, we show transcript level patterns of the four polymorphic *AOX* genes (*AOX1.1*, *AOX1.2*, *AOX2.1*, and *AOX2.2*) in percentage of total AOX accumulation in the two quinoa genotypes QQ056 and 37TES. In general, quinoa plants are salt tolerant, but the selected varieties were described to differ in the degree of salt tolerance, and the first profile belonged to the more tolerant genotype QQ056.[6] At initiation of the experiment at 0 hour, we observed similar *AOX* transcript level patterns for both varieties. However, although *AOX2.2* was the most expressed gene in both genotypes, *AOX1.1* indi-



Origin	control	8 hpi	8 hpi	24 hpi	24 hpi
D01	Polymorphism (%)	SARS-CoV-2 Wt	SARS-CoV-2 Mu	SARS-CoV-2 Wt	SARS-CoV-2 Mu
Position 1	G/A: 72/28	50/50	57/43	73/27	52/48
Position 2	T/C: 0/100	59/41	54/46	52/48	49/51
Position 3	T/C: 100/0	53/47	46/54	57/43	56/44
Position 4	C/T: 0/100	38/62	51/49	55/45	46/54
Position 5	T/C: 100/0	41/59	38/62	46/54	43/57
Position 6	G/A: 30/70	50/50	51/49	65/35	60/40
Position 7	G/A: 100/0	58/42	57/43	61/39	49/51
Position 8	G/C: 67/33	60/40	48/52	69/31	59/41
Position 9	G/A: 83/17	100/0	100/0	100/0	100/0
Position 10	T/-: 100/0	100/0	100/0	100/0	100/0
Position 11	C/A: 86/14	100/0	100/0	100/0	100/0
D03					
Position 1	G/A: 47/53	52/48	72/28	49/51	47/53
Position 2	T/C:0/100	0/100	0/100	0/100	0/100
Position 3	T/C: 100/0	100/0	100/0	100/0	100/0
Position 4	C/T: 0/100	0/100	0/100	0/100	0/100
Position 5	T/C: 100/0	100/0	100/0	100/0	100/0
Position 6	G/A: 21/79	29/71	38/62	49/51	44/56
Position 7	G/A: 100/0	98/2	100/0	100/0	100/0
Position 8	G/C: 60/40	69/31	38/62	59/41	38/62
Position 9	G/A: 100/0	100/0	100/0	100/0	100/0
Position 10	T/-: 100/0	100/0	100/0	100/0	100/0
Position 11	C/A: 59/41	59/41	74/26	59/41	60/40

B

Figure 1. Early control of stress-induced transcript level changes of polymorphic marker genes involved in ROS/RNS balancing. (A) Common control of early transcript level changes of polymorphic *AOX* genes in two quinoa varieties (QQ056 [salt-tolerant] and 37TES [less salt-tolerant]). (B) Differential control of early transcript level changes of polymorphic ASMTL genes in SARS-CoV-2-infected human NECs from two individual cell origins (wt: originally identified SARS-CoV-2; mu: SARS-CoV-2 variant; blue arrows indicate differences between cell origins; red arrows mark changes at 8 hpi; red numbers point to differences between virus variants; black arrows mark positions that discriminates the profiles at 24 hpi from the control at 0h; blue sun with yellow or blue nucleus indicate created differences between cell origins at the same time point, i.e., at 8 or 24 hpi). AOX, alternative oxidase; ASMTL, human N-acetylserotonin methyltransferase-like gene; hpi: hours post infection; NEC, nasal epithelial cell; ROS/RNS, reactive oxygen species/reactive nitrogen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

cated higher transcript levels in relation to *AOX2.2* in QQ056. Despite these individual characteristics, at 2 h both *AOX1* genes (*AOX1.1* and *AOX1.2*) increased in detriment to *AOX2.2,* whereas at 24 h the *AOX2.2* returned to increase in an individual manner. It is striking that in both genotypes the pattern of transcript level organization is almost identical at 2 h after the stress was set by salt. This strict control and coordination (*Gleichschaltung*) seems to be relieved again at 24 h. It is important to notice that higher *AOX1* accumulation at 2 h also indicates this time point as crucial to salt stress response because *AOX1* is well known as a pivotal stress-related gene in several angiosperm species. In addition, given the similar *AOX* expression pattern between contrasting varieties, we suspect that the critical differences for stress tolerance between genotypes could be related to AA or regulatory gene sequences in the same AOX isoenzyme.

Currently, we pointed to time-dependent allelic polymorphisms in one position of the human gene sequence of ASMTL during early metabolic reprogramming upon Influenza H3N2 and SARS-CoV-2 infections in NECs. We suspected that these can diversify the individual response.[2,3,5] In the coding sequence of ASMTL, we identified now 11 positions that appeared to be polymorphic due to differential transcription preferences when studying the effects of both virus types in a small number of genotypes. In Figure 1B, we present, as an example, changes observed in these 11 positions through differential polymorphic sequence transcription (in percentage of total reads per kilobase of transcript, per million mapped reads [RPKM]) at 8 and 24 hours after infection (hpi) with two SARS-CoV-2 variants (wild type [wt] and mutant [mu]) for NECs from two donor cell origins (D01 and D03). D01 cells had shown distinct performance by delayed SARS-CoV-2 virus replication in comparison with both other cell origins.[2]

In Figure 1B, it can be seen that both cell origins showed the same percentage of allele transcript levels in 9 of the 11 polymorphic positions at initiation of the experiment (blue arrows point to differences in two positions). However, in D01 cells, the transcription of diverse ASMTL gene sequences changed dramatically at 8 hpi (red arrows), and this could be reproducibly observed for cells infected by both SARS-CoV-2 variants in seven of the 11 positions. In contrast, for D03 cells, no change in the pattern of diverse ASMTL transcripts was observed for the wt SARS-CoV-2 virus, and changes due to the virus mutant occurred in four positions (percent shown in red). As a result, at 8 hpi now in seven (wt), respectively eight (mu), positions of the 11, the percentage of allele transcript levels was different between both cell origins (differential positions between D01 and D03 marked by blue suns with central yellow dots). This situation remained similar at 24 hpi (black arrows indicate differential patterns at 24 hpi compared to the control). Consequently, differences between D01 and D03 cells were observed at 24 hpi with SARS-CoV-2 wt and mutant also in eight positions (marked by blue suns with central blue dots).

We concluded from our studies across plants and human cells that efficient *Gleichschaltung* of highly diverse gene sequences of critical marker genes for adaptive ROS/RNS equilibration early upon changing environments might be a reasonable common strategy to confront disturbing situations right from the beginning with high impact for resilient behavior. ROS balancing is seen as a central mechanism in general physiology and pathophysiology, both in plants and human cells.[10,11] In view of viral infections, it might be of interest that itaconates were actually revealed as promising immune-modulatory and antiviral interventions for influenza virus infection and all three tested itaconates were reported to reduce the level of ROS.[12] In addition, Nagy and Lin[13] pointed to common antiviral strategies in plant and human cells that target the fermentation pathway. A link between stress-induced aerobic fermentation and early transcript levels of ASMTL in human cells under viral infections and of AOX activities in plant cells had been highlighted by Costa et al.[1–3] and Bharadwaj et al.[8] We released the present novel observations early after having advanced our insights in the hope that they might stimulate validation by a broader scientific effort and in diverse experimental systems.

Furthermore, we deduced from these observations that they supported our initial hypothesis that sequence diversity in marker genes for adaptive ROS/RNS equilibration could be an important trait per se for genotype-dependent and individual resilience prediction. Thus, we suggest that sequence diversity in plant AOX and human ASMTL should be explored for its potential to discriminate individual genotypes[14,15] and for its effect on supporting early diagnosis of individual capacity for relevant adaptive robustness.

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