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Impact of abiotic stressors on oleic acid accumulation in the leaves of young quinoa plants

Investigating the impact of not only individual stress factors but also their combined effect on plants becomes imperative in the context of natural and climatic fluctuations. 18-carbon unsaturated fatty acids such as oleic (18:1), linoleic (18:2) and α-linolenic (18:3) serve as vital non-enzymatic antioxidants, making significant contributions to plant defense. Moreover, they are indispensable in assessing the nutritional and biological value of plant lipids. Currently, *Chenopodium quinoa* L., a member of the Amaranth family, is increasingly recognized as a valuable source of antioxidant metabolites. Current study examined the fluctuation patterns in oleic acid ester content, serving as a non-enzymatic antioxidant, when plant exposed to varying intensities of osmotic, saline, and combined stress. A constant concentration of oleic acid esters was shown under different levels of saline stress. Osmotic stress did not affect the oleic acid content. Under salt stress, the oleic acid content increased compared to the control, varying at different NaCl concentrations. However, under combined stress, there was a significant increase in ester content, peaking at a stress level of 200NaCl/L+PEG, followed by a decrease as stress increased. It was noted that signs of stress for the photochemical quenching index of YII and ETR in photosystem II of young quinoa plants also occur with a combination of exposure to 300 mM/L NaCl + PEG. It was suggested that the level of combined stress at 200NaCl/P is transitional from eustress to distress. The results obtained could potentially become the basis for the targeted synthesis of valuable plant antioxidants for food and pharmaceutical purposes in the future.

Keywords: oleic acid, salt stress, abiotic, quinoa, eustress.

Introduction

In the context of global climate change, extensive desertification, and land salinization, plants are exposed to various abiotic stressors. Generally, abiotic stressors exert their impact concurrently by altering osmotic pressure, disrupting nutrient supply, inducing ion toxicity, and causing oxidative damage to cells and tissues [1–3]. Comprehension of above stressors is complicated due to their complexity, including source, intensity, duration, and effects [4].

Nature has evolved diverse mechanisms to prevent or alleviate abiotic stress, which encompasses a powerful antioxidant defense system consisting of both enzymatic and non-enzymatic components. In addition, 18-carbon unsaturated fatty acids (UFAs), including oleic (18:1), linoleic (18:2), and α-linolenic (18:3) acids, serve as vital non-enzymatic antioxidants, contributing significantly to plant protection [5, 6].

Furthermore, UFAs serve multiple roles within plant tissues, specifically they: a) function as constituents and regulators of cell membranes in glycolipids; b) serve as carbon and energy reserves within triacylglycerol; c) govern the storage of extracellular barrier components like cutin and suberin; d) act as precursors to various biologically active molecules, including jasmonates and nitroalkenes; e) function as regulators in stress signaling pathways, while also having the potential to induce oxidative stress [5, 7].

The alterations in lipid composition that occur during a plant's adaptation to adverse environmental conditions can determine the types of radical-free reactions induced by stress [8]. Fatty acids in plants exist not only in their free form but also as esters [9].

Throughout history, humans have utilized plants for nutrition and medicinal purposes since plant antioxidants possess the ability to regulate not only the physiological processes within plants but also various functions within the human body, reducing the risk of chronic diseases caused by free radical oxidation [10, 11]. Consequently, understanding the antioxidant systems of food and medicinal plants holds great significance. The primary reference point for assessing the nutritional and biological value of plant lipids is UFAs content [12].

Epidemiological research suggests that a diet with a higher proportion of UFAs, particularly oleic acid, may offer protection against cardiovascular disease. Oleic acid is acknowledged for its exceptional resistance to oxidation and its capacity to enhance the activity of other antioxidants, such as tocopherol [13].

Nowadays, plants from the *Amaranthaceae* family, particularly quinoa (*Chenopodium quinoa* L.), have been gaining heightened recognition as valuable sources of antioxidant metabolites [14, 15]. Additionally, amaranth species are stress-resistant and well adapted for cultivation in marginal regions [16–18].

Therefore, current research aimed at investigating the accumulation of oleic acid in quinoa plants when subjected to osmotic, saline, and combined stress conditions.

Materials and Methods

Plant material

The study utilized the Tajik quinoa variety "Vandat" obtained from the Centre for Genetic Resources of the Tajik Academy of Agricultural Sciences. The experiment involved plants with no cotyledons and four rows of unfolded leaves. Two top unfolded leaves and the intervening stem section were examined.

Growth conditions

Research plants were grown in a climatic chamber with fluorescent lamps providing 200 μmol m^{-2} s^{-1} PAR, a 16-h photoperiod, and a temperature of +25 °C. Seeds were germinated for 5 days, and then transplanted into plastic pots (20 seedlings pot^{-1}). Seedlings were exposed to circadian illumination for 10/14 h. The seedlings were cultivated for 26 days using 50 % Hoagland nutrient solution, and for the next 14 days with added stress agents, resulting in a total of 8 experimental conditions (Table 1).

Table 1

Experimental conditions

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
	Control	P	100NaCl	200NaCl	300NaCl	100 NaCl/P	200NaCl/P	300NaCl/P
Days	14	10 + 4	14	14	14	10 + 4	10 + 4	10 + 4
50 % Hoagland solution	+	+	+	+	+	+	+	+
PEG-6000	-	12.5 % (m/v)	-	-	-	12.5 % (m/v)	12.5 % (m/v)	12.5 % (m/v)
NaCl	-	-	100 mM	200 mM	300 mM	100 mM	200 mM	300 mM

Determination of organic compounds in extracts

Gas chromatography with mass spectrometric detection (Agilent 6890 N/5973 N, Santa Clara, CA, USA) was employed to analyze organic compounds. Plant samples were fixed in 96 % ethanol at a ratio of 100 g of tissue to 500 mL of ethanol. Extraction occurred in an orbital shaker across two stages until a clear and colorless solvent was obtained. A 1.0 μL sample was injected into the GC-MS system at 260 °C without flow division. The separation utilized a DB-35 MS chromatographic capillary column with a constant carrier gas velocity of 1 mL min^{-1}. The chromatographic temperature rose by 10 °C min^{-1} from initial 40 to 150 °C, followed by 5 °C min^{-1} rate from 150 to 300 °C. Detection was performed using SCAN m/z 34–850 mode. GC system regulation and results processing was carried out by Agilent MSD ChemStation software (Santa Clara, CA, USA).

Photosynthetic Activity Determination

Photosynthetic activity parameters were estimated by determination of fluorescence levels. Rapid light curves (RLCs) were recorded using Junior-PAM ("Heinz WalzGmbH", Effeltrich, Germany) under actinic illumination of 450 nm. The RLC for each sample was recorded after quasi-darkness to assess the effect of actinic light absence, while complete darkness is difficult to achieve under field conditions [19]. For each measurement the fluorometer provided eight saturation light pulses of 10,000 μmol/m^2 s every 20 s, while actinic light increased from 0 to 625 μmol/m^2 s gradually. For comparison, the data obtained from the last pulse of the light curve were taken [20]. The following parameters were calculated using WinControl-3.29 (Walz, Effeltrich, Germany) software: Y(II): effective photochemical quantum yield of PSII; ETR: PSII relative electron transport. In the experiment, each time the region of the middle third of the active leaf was selected. All measurements were performed on a sunny day from 09:00 to 11:00 a.m.

All experiments were done in three replicates. The processing of data and graphing was performed using Microsoft Excel (Microsoft Corp., Redmond, Washington, DC, USA). Atypical values were excluded from the data based on t-tests, the standard error of the average sample was calculated. Differences were considered significant at $p < 0.05$.

Results and Discussion

Oleic acid, a monounsaturated carboxylic acid, is characterized by a single double bond. The systematic name of oleic acid is 9-octadecenoic acid with chemical formula of $CH_3–(CH_2)_7–CH=CH–(CH_2)_7–COOH$. Oleic acid is an oily, colorless, and odorless liquid, with a density lower than that of water. It is insoluble in water but exhibits solubility in organic solvents. The melting point of oleic acid is +13.4 °C, and its empirical formula is $C_{18}H_{34}O_2$ **(**Fig. 1**)**.

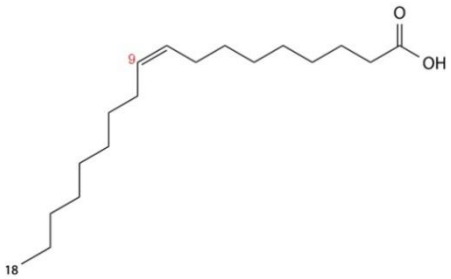

Figure 1. Oleic acid chemical structure

The examination of oleic acid ester content in the leaves of experimental quinoa plants revealed significant quantitative variations as stress levels of osmotic, saline, and combined nature increased (Table 2).

Table 2

The influence of stress conditions on oleic acid ester content in photosynthetic organs of young *Chenopodium quinoa*.

Condition	Control	+100 mM NaCl	+200 mM NaCl	+300 mM NaCl
Saline stress	3.13 ± 0.06	5.02 ± 0.03*	5.00 ± 0.02*	5.01 ± 0.03*
Combined stress (+12.5 % PEG)	3.03 ± 0.05	5.55 ± 0.06*	11.3 ± 0.08*	6.67 ± 0.06*
Note – * indicates a significant difference between the control and the stress at $p \leq 0.05$				

Thus, individual osmotic stress did not induce alterations in the oleic acid ester content. Conversely, under individual saline stress, the oleic acid ester content increased compared to the control but remained consistent with increasing NaCl concentration.

The picture became more complicated under combined stress conditions. The tendency for oleic acid ester content to increase compared to control persisted, with the highest levels observed at combined stress of 200NaCl/P. However, at a combined stress level of 300NaCl/P, there was a reduction in oleic acid ester content, even though remaining higher than the control values.

Consequently, stress reactions can significantly alter the UFAs content and shape the lipid composition of plant cells and tissues during the adaptation process [21, 22].

Since fatty acids can only undergo *de novo* synthesis within plastids, specifically in chloroplasts [4, 23], the increased content of UFAs, including in various ester forms, within the lipids of internal chloroplast and mitochondria membranes can serve as natural antioxidants, thereby potentially contributing to the attenuation of PSII photoinhibition under stress conditions [24, 25].

Our analysis of YII, a key indicator of photochemical quenching that evaluates the effective photochemical quantum yield of PSII, did not reveal any statistically significant variations under stress conditions. However, it revealed a trend of increasing values with the rise in NaCl concentration in the nutrient solution up to 200 mM/L, followed by a marked decrease at a concentration of 300 mM/L NaCl under combined stress. Literature suggests that consistent photochemical quantum yield values indicate PSII's resilience to saline conditions [26], whereas a decrease signifies photodamage caused by stress [27, 28]. Consequently, it appears that stress indicators related to photochemical quenching in PSII of young quinoa plants become ev-

ident under the combined effects of 300 mM/L NaCl and PEG6000. This phenomenon has been similarly documented in quinoa and various other plant species [29–31] (Fig. 2).

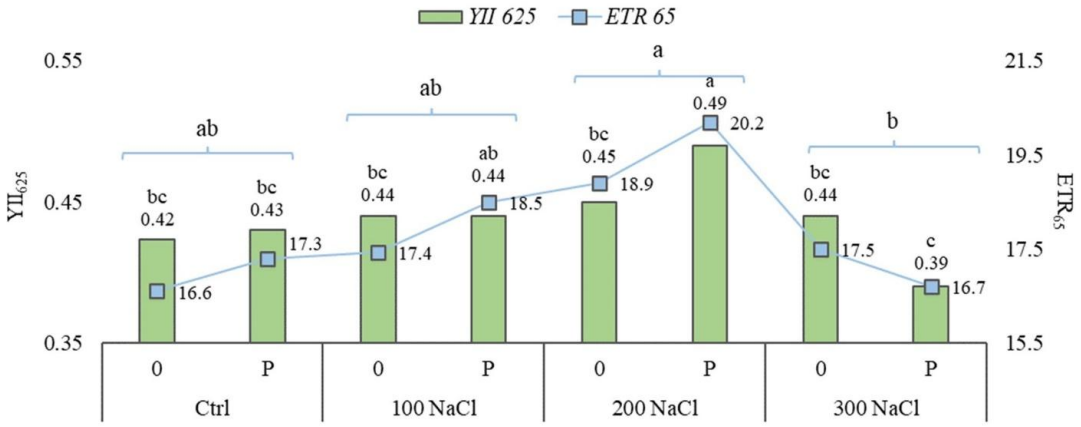

Figure 2. Activity alterations of photosynthetic parameters under stress conditions. Different letters above the bars represent significant differences at $p \leq 0.05$

The negative effects of combined stress on the PSII quantum efficiency index likely arise from the inhibition of electron transport, disruption of reaction centers, and the initial stages of damage to the oxygen-evolving complex [30, 32, 33]. In our study, we observed an uptick in the electron transport rate (ETR) as the concentration of NaCl in the nutrient solution increased to 200 mM/L, under both salt and combined stress conditions. This increase correlated with the leaf's water content, whereas a significant decrease in ETR was seen at a NaCl concentration of 300 mM/L. Under salt stress alone, ETR values returned to baseline (control) levels, but they were markedly lower under combined stress. These observations are in line with research suggesting that PSII-mediated electron transport can improve under mild salinity [34] but is adversely affected under intense stress [35], leading to diminished electron transport capacity during photosynthesis and reduced efficiency in energy utilization from PSII [35, 36]. The disruption of intersystem electron transport and damage to PSII's terminal electron acceptors play significant roles in generating reactive oxygen species [37]. This indicates that young quinoa plants facing severe combined stress experience greater oxidative stress compared to those under normal conditions or dealing with only osmotic or salt stress. A strong correlation (r = 0.9) between YII625 and ETR values further supports the conclusion that genuine stress symptoms in young quinoa plants manifest under the combined influence of osmotic and salt stress at a 300 mM/L NaCl +PEG concentration.

The disruption of photosynthesis is one of the earliest responses to stress [38–40] and consistent high levels of oleic acid esters across different saline stress levels may suggest a noteworthy degree of salt tolerance for photosynthetic organs in young quinoa plants. This aligns with previously published findings on the morphological and anatomical responses of quinoa's photosynthetic tissues [41].

The significant elevation in oleic acid ester content observed at 200NaCl/P, followed by a decline as stress levels increase to 300NaCl/P, may potentially indicate that the stress level of 200NaCl/P signifies a transition from eustress to distress for young quinoa plants. During eustress, all physiological processes are geared towards growth and development. Contrarily, during distress, all plant adaptive mechanisms turn off [26].

Conclusion

Thus, the study findings represent a new step towards a better understanding of adaptation mechanisms in quinoa plants exposed to individual and combined stress. This could potentially pave the way for future advancements in the targeted synthesis of valuable plant antioxidants for pharmaceutical purposes by focusing on specific stressor concentrations on plants possessing significant nutritional and medicinal attributes at distinct developmental stages.

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Абиоттық стрессорлардың жас киноа өсімдерінің жапырақтарында олеин қышқылдарының жиналуына әсері

Табиғи-климаттық өзгерістер құбылысында өсімдіктерге жалғыз ғана емес, сонымен қатар біріктірілген күйзеліс факторларының әсерін зерттеу маңызды. Олеин (18:1), линол (18:2) және α-линолен (18:3) сияқты 18 көміртекті қанықпаған май қышқылдары өсімдік қорғанысына маңызды үлес қосатын ферментативті емес өмірлік маңызды антиоксиданттар ретінде қызмет етеді. Сонымен қатар, олар өсімдік липидтерінің тағамдық және биологиялық құндылығын бағалауда өте қажет. Қазіргі таңда Амарант тұқымдасына жататын *Chenopodium quinoa* L. өсімдігі антиоксиданттық метаболиттердің құнды көзі ретінде жақсы танылуда. Мақалада жас киноа өсімдіктерінің фотосинтезге қабілетті мүшелерінің осмостық, тұзды және әртүрлі қарқындылықтағы аралас күйзелістер кезінде ферментативті емес антиоксидант ретінде олеин қышқылы күрделі эфирлерінің құрамының өзгеру динамикасы зерттелген. Әртүрлі деңгейдегі тұзды стрестің әсерінен олеин қышқылы тәрізді күрделі эфирлерінің тұрақты концентрациясының көтерілгені көрсетілді. Тұзды стресс жағдайында олеин қышқылының мөлшері әртүрлі NaCl концентрацияларында өзгеріп, бақылаумен салыстырғанда жоғарылады. Дегенмен, біріктірілген стресс кезінде күрделі эфир құрамының айтарлықтай артуы байқалды, ол 200NaCl/L+PEG стресс деңгейінде максимумға жетіп, содан кейін стресс жоғарылаған сайын азаяды. Жас киноа өсімдіктерінің II фотожүйесінде YII және ETR фотохимиялық сөндіру индексі үшін стресс белгілері 300 мМ/л NaCl + ПЭГ әсерінің комбинациясы кезінде де артатыны атап өтілді. 200NaCl/P кезінде біріктірілген стресс деңгейі

эустрестен күйзеліске ауысады деген болжам бар. Алынған нәтижелер болашақта тағамдық және фармацевтикалық мақсаттарға арналған құнды өсімдік антиоксиданттарының мақсатты синтезіне негіз бола алады.

Кілт сөздер: олеин қышқылы, тұзды стресс, абиотикалық, киноа, эустресс.

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Влияние абиотических стрессоров на накопление олеиновой кислоты в листьях молодых растений киноа

На фоне природно-климатических изменений актуально изучение влияния на растения не только одиночных, но и комбинированных стрессоров. 18-углеродные ненасыщенные жирные кислоты, такие как олеиновая (18:1), линолевая (18:2) и α-линоленовая (18:3), служат жизненно важными неферментативными антиоксидантами, внося значительный вклад в защиту растений. При этом они являются незаменимыми при оценке пищевой и биологической ценности растительных липидов. В настоящее время представитель семества Амарантовых *Chenopodium quinoa* L. получает все большее признание как ценный источник антиоксидантных метаболитов. В статье рассмотрена динамика изменений содержания эфиров олеиновой кислоты как неферментативного антиоксиданта при осмотическом, солевом и комбинированном стрессах различной интенсивности в фотосинтетических органах молодых растений киноа. Показаны увеличение и неизменная концентрация содержания эфиров олеиновой кислоты при различных уровнях солевого стресса. В условиях солевого стресса содержание олеиновой кислоты увеличивалось по сравнению с контролем, варьируя при разных концентрациях NaCl. Однако при комбинированном стрессе наблюдалось существенное увеличение содержания эфиров, достигающее максимума при уровне стресса 200NaCl/L+ПЭГ, за которым следовало снижение по мере усиления стрессового воздействия. Отмечено, что признаки стресса для показателя фотохимического тушения YII и ETR в фотосистеме II молодых растений киноа также наступают при сочетании воздействия 300 mM/L NaCl+ПЭГ. Сделано предположение о том, что уровень комбинированного стресса в 200NaCl/P является переходным от эустресса к дистрессу. Полученные результаты потенциально могут стать основой для направленного синтеза ценных растительных антиоксидантов для пищевых и фармацевтических целей в будущем.

Ключевые слова: олеиновая кислота, солевой стресс, абиотический, киноа, эустресс.

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