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The Screening of Water Stress Tolerant Wheat Cultivars with Physiological Indices

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ABSTRACT

Development and cultivation of drought tolerant cultivars is perquisite for wheat production under water deficit condition. Many new winter wheat varieties with high yield and good quality have been released and extensively cultivated in Winter Wheat Irrigation Region of China in recent years. However, if these cultivars are adapt to arid land need to be investigated. In the present study, five newly released elite winter wheat cultivars Xiaoyan22, Shaan354, Changwu134, Chang4640, Xinong928, including two reference cultivars Shanhe6 and Zhengyin1with strong drought resistance and sensitiveness respectively, were used for assessment of water stress tolerance by three physiological indices i.e., relative water content (RWC), malondialdehyde (MDA), and free proline content(PC). The results showed that Changwu134 and Xinong928 had least reduction in leaf RWC, least increase in leaf MDA content and largest amount of PC accumulation under 48 or 72 h of water stress. While Xiaoyan22 exhibited greatest reduction in leaf RWC, largest increase in leaf MDA content and lowest amount of PC accumulation. The RWC and MDA content of Changwu134 and Xinong928 of stress treated group reached to the control level when stress was dismissed for 24 h, while those of Xiaoyan22 did not. Combining the three physiological parameters, the rank of water stress tolerance of these cultivars is: Xinong928>Shaanhe6>Changwu134>Chang4640>Zhengyin1>Shaan354>Xiaoyan22. Xinong928 is most drought resistant cultivar among the newly released wheat cultivars. The results provided important information for extending these new wheat cultivars to water deficient area.

Key words: Drought stress, Malondialdehyde, Polyethylene glycol, Proline, Relative water content, *Triticum aestivum* L.

Introduction

Wheat is the staple food for more than 1/3 of the world's population, and crop yield is significantly influenced by global climate changing and limitation of water resources in the environment (AL-Ghamdi, 2009). Drought, one of the environmental stresses, is a worldwide problem that constraining plant growth and limiting the global crop production seriously, in majority of agriculture fields of world and recent global climate change has made this situation more adverse (Abedi and Pakniyat, 2010).

Drought affects morphological, physiological, biochemical and molecular processes in plants resulting in growth inhibition, stomatal closure with consecutive reduction of transpiration, decrease in chlorophyll content and inhibition of photosynthesis and protein biosynthesis. The extent of these changes is dependent on the time, stage and severity of environmental stress (Hong-Xing *et al*., 2011). Plant experiences dehydration under various unfavorable environmental conditions such as high soil salinity, low temperature and water deficit conditions. To counteract such adversaries plants tend to adopt a variety of defense protective mechanisms (Holmstrom *et al*., 2000). Maintenance of plant water status is a fundamental phenomenon for normal growth of plants under stressful environment. Disturbances in water balance in plants lead to impaired functioning of different gas exchange attributes, ultimately resulting in reduced plant growth (Demirevska *et al*., 2010; Rapacz *et al*., 2010). Under such conditions, plants generally accumulate some kind of compatible solutes such as proline, betaine and polyols in the cytosol to raise osmotic pressure and thereby maintain both turgor and the driving gradient for water uptake (Rhodes and Samaras, 1994) and to protect membranes and proteins.

It has been shown that proline has a key role in stabilization of cellular proteins and membranes in presence of high concentration of osmoticum (Errabii *et al*., 2006). Vendruscolo *et al*., (2007) found that proline is involved in tolerance mechanism against oxidative stress and this was the main strategy of plants to avoid detrimental effects of water stress. Free proline content for plants normally supplied by water is 0.2 to 0.6mg/g dry matter, while during water stress may reach to 40-50mg/g dry matter. Increased proline in the stressed may be an adaptation to overcome the stress condition (Chandrashekar and Sandhuarani, 1996).

Many researches have indicated that the accumulation of malondialdehyde (MDA), a product of fatty acid peroxidation, when plants are exposed to abiotic stress including drought, salt, cold stress conditions, suggesting

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serious membrane damage and plants' status is being disturbed. The MDA accumulation in plants due to cellular membrane lipid peroxidation is a measure of oxidative stress induced membrane damage during water stress (Farooq *et al*., 2010).

Water stress causes water losses within the plant and resulted in relative water content (RWC) reduction. In this sense, one of the most reliable and widely used indicators for defining both the sensitivity and the tolerance to water deficit in plants is leaf RWC (Rampino *et al*., 2006; Sanchez-Rodriguez *et al*., 2010).

The search for indices which can show plants water stress tolerance is an important step in the selection of wheat plants with desired performance under conditions of limited water supply. Field experiments investigating the yields of different cultivars under water deficit conditions are the most reliable way to assess their drought tolerance. However, it takes 2-3 years to get results for winter wheat. In addition，a very large area is essential if many cultivars are evaluated at the same time. There for, the methods for assessing water stress tolerance of wheat plants at seedling stage in Laboratory have been developed. For example exposing the root system of plants to osmotic solutions has been used to impose water stress on plants for investigation of drought tolerance (Agarwal *et al*., 1994). Several methods which range from withdrawal of water to the use of chemicals such as polyethylene glycol, mannitol etc., have been employed to create water stress in plants (Emmerich *et al*., 1990). Among these methods, polyethylene glycol 6000 (PEG6000) was most widely used to introduce water stress to the hydroponically grown plant seedlings (Lu and Neumann, 1998). Plant Physiological indices under water stress conditions, including RWC, MDA accumulation, proline content (PC) ect, have been extensively used to assessing plant drought tolerance.

Development of drought tolerant varieties is perquisite for better crop production, yield improvement and yield stability under water deficit condition (Siddique *et al*., 2000).With the development of wheat breeding methods and techniques, more than 50 winter wheat varieties with high yield and good quality in irrigation regions are released. In the recent 10 years, these wheat varieties are extensively cultivated in Winter Wheat Region of China. Among these cultivars, Xiaoyan22, Shaan354, Xinong928, Changwu134 and Chang4640 are most elite winter wheat cultivars extensively planted in the irrigation area of Winter Wheat Region of China (Huang-Huai). However, the tolerance of these cultivars under water deficit conditions is still unknown, and if they can also produce high yields in arid or semi-arid winter wheat region need to be investigated. The drought tolerance evaluation of these cultivars was the first priority before their application to non- irrigation regions.

In the present study, drought tolerance of five newly released elite winter wheat cultivars was assessed by three physiological indices, RWC, MDA, PC, using two well-known old cultivars with strong drought resistance and sensitiveness as references. Water stress condition was induced by treating the roots of wheat seedlings with polyethylene glycol (PEG6000). The supreme goal of current study is to screen drought resistant wheat cultivars from these newly released elite wheat types, providing the basic information for enhancing wheat production by extensive cultivation of newly released drought resistant wheat cultivars in arid or semi-arid winter wheat region.

Materials and Methods

Plant materials:

Seven wheat cultivars, including five newly released elite wheat cvs., Xiaoyan22, Shaan354, Changwu134, Chang4640, Xinong928, and two wheat cvs., Shaanhe6 and Zhengyin1 (released 30 years ago), as drought resistance and sensitiveness reference cultivars respectively, were used to assess drought resistance or tolerance of aforementioned cultivars. All the seeds of these cultivars were provided by Wheat Research Center, Northwest A & F University.

Plants growth conditions and sample collection:

Seeds of each cultivar were germinated under hydroponic conditions and grown in greenhouse with a day/night temperature regime of 20–22 ^{o}C/15–18 ^{o}C，65-75% relative humidity and a light period of 16 h/day, regulated with supplementary light, Six trays (40-50 plants/per tray) for each cultivar. The Hoagland solution, a hydroponic nutrient solution that was developed by Hoagland and Snyder in 1993 and contains all necessary nutrients for normal plant growth, was supplied for wheat growth, and was aerated using an air compressor. At fully developed two leaves stage (about 14 days old), plants of each cultivar were divided into two groups, each group including three trays. One group as control plants normally supplied with the Hoagland solution, while another group as drought treatment group supplied with the Hoagland solution containing 20% PEG 6000 for three days and then normally supplied with the Hoagland solution again (rewater-Rw).

The leaf samples of each cultivar were randomly collected from control or drought treatment groups, at the same time point during the stress period (24, 48, and 72 h), and re-water period (24 and 48 h). The experiment was performed triplicate dependent biological repeat. The collected leaf samples were used for the estimation of RWC, MDA and PC.

Estimation of RWC of leaves:

Leaf relative water content (RWC) was estimated according to the method of Smirnoff, (1993).

Estimation of PC of fresh leaves:

The proline content (PC) was measured by the method of Bates *et al*., (1973).

Estimation of MDA contents in leaves:

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content, a product of lipid peroxidation following method of Hodges *et al*., (1999).

Statistical analysis:

Results were based on mean values of at least three replicates from two (independent) repeats of experiment. The mean values were then compared using Duncan's multiple range test at P=0.05. MSTATC computer software was used to carry out statistical analysis (Bricker, 1991).

Results:

Leaf RWC of different wheat cultivars under water stress:

Our experimental data (Table1) suggested that all the wheat cultivars maintained high RWC under normal water supply. When exposure to water stress for 24 h, all cultivars had no significance difference in the leaf water status compared with their control, with the exception of Zhenyin1 and Chang4640 which had a significant decline at this time point. All cultivars except Zhengyin1 and Xiaoyan22 showed a mild decrease in their leaf RWC after 48 h of water stress. The leaf RWC of all wheat cultivars become declined more significantly when the water stress was prolonged. Xiaoyan22, Zhengyin1, Shaan354 and Chang4640 exhibited much more decrease in leaf RWC, especially in case of Xiaoyan22 which showed 46.31% decreased. Water deficit condition caused more or less reduction in leaf water status of almost all cultivars. This depicted that wheat cultivars possess various ability of maintaining their RWC under both water deficit and well-watered conditions. Zhengyin1 and Xiaoyan22 showed continuous and great reduction in their water status during 3 days of water stress period. The decline in leaf water was not found to be significant in Shaanhe6, Changwu134, and Xinong928, because it showed a mild decrease and then maintained it through out the stress period.

In treatment group, the leaf RWC of Changwu134, Xinong928 and Chang4640 increased near to their control level after 24 h re-watering, while that of Zhenyin1, Xiaoyan22, Shaanhe6, and Shaan354 still had significant differences from their controls. Leaf RWC of each wheat cultivars in treatment group increased to the level of their controls after 48 h re-watering, with the exception of Xiaoyan22 which is still significantly less than the control level.

So the order of drought tolerance of these wheat cultivars under water stress condition according to leaf water status is: Changwu134>Xinong928> Shaanhe6> Chang4640> Zhengyin1> Shaan354 > Xiaoyan22. These suggested that Xinong928 and Changwu134 are most water stress tolerant cultivars among all the investigated wheat cultivars, and Xiaoyan22 most water stress sensitive.

Leaf free PC of different wheat cultivars under water stress:

The endogenous proline contents in the leaves of each wheat cultivar were monitored in both control and water stressed plants (Fig. 1). Only low amount of leaf free PC was detected under normal water regime. All cultivars except Zhengyin1, exhibited a significant increase in PC in 24 h of water stress, especially in changwu134. The increase of leaf PC in all cultivars excluding Zhengyin1 became more apparent with prolonged time of water stress. Changwu134 showed much rise in leaf PC compare to other wheat cultivars during the first 2 days of water stress period. However, the increase was much more in Xinong928 (78.81%), Changwu134 (62.18%) and Shaan354 (57.36%) and least in Zhengyin1 (12.19%) under 72 h of water stress, compared with their control, indicated that Xinong928 had the highest amount of leaf PC. It was noted that no significant increase in Leaf PC of treated group of zhengyin1 as compare to control was detected throughout the whole experiment. After 24-48 h recovery from water stress, the PC in leaves of each cultivar was almost declined to normal level.

Table 1: Leaves RWC (%) of seven wheat cultivars under stressed and unstressed conditions (CK).

Wheat Cultivars[a]	Treatment time (hrs)[b]					
	Ck	24hrs	48hrs	72hrs	24hrs-rw	48hrs-rw
Zhengyin1 (1.993)	96.56 A ±1.51	93.40 B ±1.73	82.47 C ±2.06	74.66 D ±3.45	93.48 B ±1.78	96.27 A ±1.81
Shaanhe6 (2.559)	94.80 A ±1.83	92.73 A ±1.68	88.40 B ±1.19	87.30 B ±1.21	89.20 B ±1.24	92.80 A ±1.49
Changwu134 (7.839)	98.26 A ±2.53	93.60 A ±2.13	93.95 A ±2.09	72.25 B ±3.54	92.10 A ±1.95	96.26 A ±2.17
Chang4640 (1.839)	96.10 A ±1.7	91.70 B ±1.21	88.20 C ±2.46	84.30 D ±3.14	94.43 A ±1.91	95.30 A ±1.65
Xinong928 (2.117)	95.73 A ±1.37	95.80 A ±1.52	92.63 B ±2.05	87.67 C ±1.56	94.46 AB ±0.24	95.08 A ±1.49
Shaan354 (2.026)	97.58 A ±1.47	97.44 A ±1.93	90.36 C ±2.23	87.00 D ±2.88	92.67 B ±1.57	95.56 A ±1.66
Xiaoyan22 (1.303)	95.88 A ±1.67	95.73 A ±1.77	84.15 D ±3.77	53.69 E ±2.72	87.78 C ±1.82	92.11 B ±1.69

[a]Values given in parenthesis is LSD of respective cultivar.

[b]Mean values with the same superscript are non-significant. Different capital letters after each mean value indicate significant difference at P < 0.05 level.

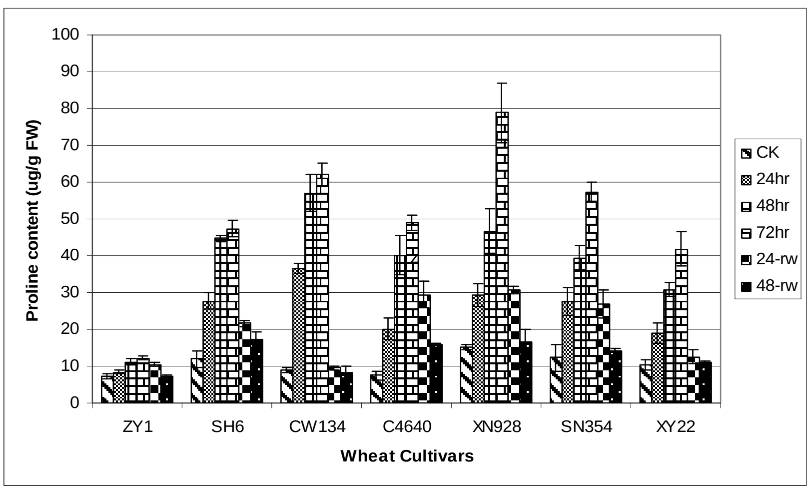

Fig. 1: Proline content comparison of 7 wheat cultivars under water stress and control treatments. Error bars show the standard error of the mean (SE). (ck; control, ZY1; Zhengyin1, SH6; Shaanhe6, CW134; Changwu134, C4640; Chang4640, XN928; Xinong928, SN354; Shaan354, XY22; Xiaoyan22).

Leaf MDA content of different wheat cultivars under water stress:

The data showed that a great increase in MDA content was observed in all genotypes except Xinong928 and Shaanhe6 in 24 h of water stress, but Shaan354, Chang4640, Xiaoyan22 and Zhengyin1 had shown much rise in MDA content in 48 h of water stress, whereas other three cultivars showed little increase. With the prolonged water stress treatment, almost all cultivars exhibited significant increase in MDA content in 72 h of water stress, except the Shaanhe6 and Xinong928. Prolonged treatment with 20% PEG had stronger effect on treated plants and disclosed more differences between the genotypes during it.

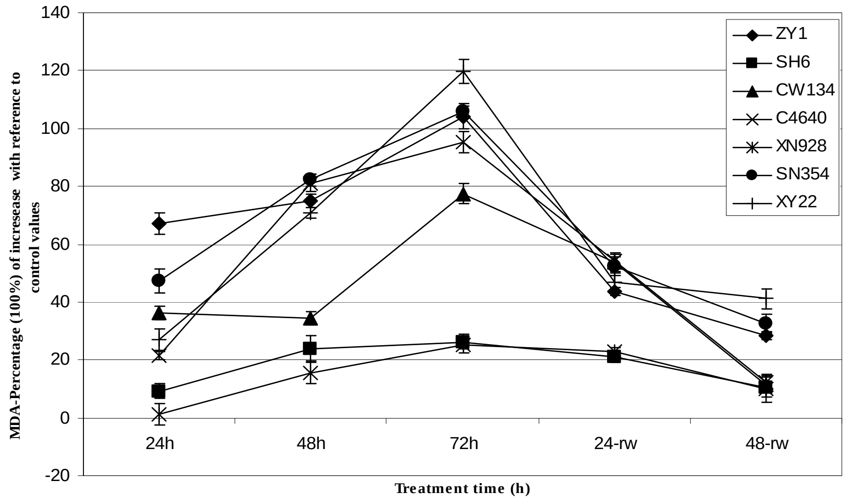

Fig. 2: Effects of water stress on membrane lipid peroxidation (malondialdehyde-MDA) in the leaves of wheat cultivars. Error bars show the standard error of the mean (SE). (ZY1; Zhengyin1, SH6; Shaanhe6, CW134; Changwu134, C4640; Chang4640, XN928; Xinong928, SN354; Shaan354, XY22; Xiaoyan22)

After water stress period, all the cultivars showed much improvement in their membrane damage at the end of 1st day water recovery. At the end of 2nd day stress recovery, lipid peroxidation membrane damage was significantly improved in almost all genotypes except Shaan354, Xiaoyan22 and Zhernyin1, who still had a high MDA content compared with their controls, indicating that these three cvs., showed maximum membrane damage and also could not well-repaired at the end of water stress recovery period. Whereas this damage was lowest in Xinong928 and Shaanhe6 which had little increase in MDA content during 72 h water stress and almost recovered at 48 h of water recovery. The order of resistance towards water stress according to the degree of lipid peroxidation membrane damage is: Xinong928>Shaanhe6>Changwu134>Chang4640>Zhengyin1> Shaan354>Xiaoyan22. This indicate that Xinong928 is most drought resistant cultivar, and Xiayan22 most drought sensitive.

In general, the order of drought tolerance of all these wheat cultivars assessed by combining the three studied physiological parameters is: Xinong928>Shaanhe6>Changwu134>Chang4640>Zhengyin1>Shaan354> Xiaoyan22.

Discussion:

In cereals it has been observed that drought resistance is a quantitative trait and RWC served as a relevant tool for the screening of drought tolerance species (Teulat *et al*., 2003). In fact, tissue RWC gives an idea of the water status of plants at specific conditions. The metabolic activity in leaf tissues can be estimated by measuring RWC which is then consider as an integrated measure of plant water status (Flower and Ludlow, 1986). The leaf RWC of all the wheat cultivars investigated in the present study maintained high RWC under normal water supply, and this was in agreement with the results reported by Rampino *et al*., (2006). When exposure to water stress for 24 h, almost all cultivars had no significance difference in the leaf water status compared with their control. The leaf RWC of all cultivars except Zhengyin1 and Xiaoyan22 showed a mild decrease after 48 h of water stress, and the RWC of all wheat cultivars declined more significantly with the prolonged water stress. Zhengyin1 and Xiaoyan22 showed continuous and great reduction in their water status during 3 days of water stress period. The reduction in leaf RWC of Shaanhe6, Changwu134, and Xinong928 was mild through out the stress period. Different water status, under water stress and irrigated regime, was also observed by many authers (Abbad *et al*.,, 2004; Sairam and Saxena, 2000; Tambussi, 2000). Water deficit condition caused more or less reduction in leaf water status of almost all cultivars investigated in this study. This elucidated that the different wheat cultivars possess various ability of maintaining their RWC under water deficit conditions. The order of water stress tolerance of all these wheat cultivars investigated in the present study according to leaf water status is: Changwu134>Xinong928>Shaanhe6Chang4640>Zhengying>Shaan354>Xiaoyan22.

According to Jones (1992), several mechanisms exist by which plants can maintain their physiological activity when they are subjected to water stress. One of these mechanisms is osmotic adjustment, which is due to the accumulation of osmotically active substances such as glycinebetaine, sorbitol and proline, in plant leaves in response to water stress treatment (Szegletes *et al*., 2000). Free proline in plants play a key role in stabilization of cellular proteins and membranes in presence of high concentration of osmoticum (Errabii *et al*., 2006). Vendruscolo *et al*., (2007) found that proline is involved in tolerance mechanism against oxidative stress and this was the main strategy of plants to avoid detrimental effects of water stress. Palfi *et al*., (1982) reported that under water stress situation, proline content can increase from 10 to 100 times. Increased proline in the stressed may be an adaptation to overcome the stress condition (Chandrashekar and Sandhuarani, 1996). However, the opinions reported on the role of proline accumulation in conferring tolerance to drought stress are somewhat controversial and it is still not clear whether or not the increase in proline levels can confer enhanced drought tolerance (Nayyar and Walia, 2003). Zlatev and Stoyanov, (2005) suggested that proline accumulation of plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism. In the present study, all cultivars chosen exhibited a significant increase in PC under 24 h of water stress, and the increase became more apparent with the prolonged time of water stress, with the exception of drought sensitive reference cultivar (Zhengyin1) which had no significant increase in Leaf PC as compare to its control throughout the entire stress period. An increase in proline accumulation during water stress has also been reported for hexaploid and tetraploid wheat (Vendruscolo *et al*., 2007), and some other plants such as rice leaves, canola leaf discs, potato leaves, maize leaves. Based on theory of Jones (1992), our results suggest that Xinong928 and Changwu134 are the most drought resistant cultivar, while Xiaoyan22 most drought sensitive.

As the plants were exposed to drought stress, the dysfunction of metabolism occurred with massive accumulation of free radicals and ROS, which would attack cell membrane by inducing peroxidation of the membrane lipids and eventually led to the damage of cell membrane and cell death (kato *et al*., 2002). MDA was the major product of membrane lipid peroxidation and its content indicated the level of membrane lipid peroxidation and the extent of membrane injury (*Zhang et al*., 2004). As indicated in figure2, the MDA content in wheat cultivars leaves increased with the prolong exposure to drought stress. Our data reflected that the MDA content was very high in leaves of Xiaoyan22, Shaan354 and Zhengyin1 under 72 h of water stress compared with their control values, while that of Xinong928 and Shaanhe6 very low, indicated that drought sensitive cultivars showed more peroxidation damage of membrane lipids compare to drought resistant ones. The same trend was observed by many workers (Moussa and Abdel-Aziz, 2008; Fazeli *et al.*, 2007; Bandurska and Jozwiak, 2010). On the other hand after water stress recovery period, again Xinong928 and Xiaoyan22 had shown highest and lowest MDA values respectively among all cultivars. In addition, Shaan354 and Xiaoyan22 exhibited some extent of recovery from water stress after re-watering but could not fully recovered from the cell membrane damage after 48 h of re-watering. This indicated that these two cultivars have not ability to maintain the cell membrane integrity and also once severely damage could not recover completely. The results are also in agreement with the findings of the study of Cunhua *et al.,* (2010) and, Xu and Zhou, (2006). Among all the wheat cultivars, the order of resistance towards water stress according to the degree of lipid peroxidation membrane damage is: Xinong928>Shaanhe6>Changwu134>Chang4640>Zhengyin1>Shaan354>Xiaoyan22. These indicated that Xinong928 is most drought resistance cultivar, and Xiaoyan22 most drought sensitive.

In general, combining the three studied physiological parameters, the order of water stress tolerance of all the wheat cultivars chosen in current study is: Xinong928>Shaanhe6>Changwu134>Chang4640>Zhengyin1> Shaan354>Xiaoyan22. Xinong928 is most drought resistant cultivar among the newly released wheat cultivars, compared with the well-known drought resistant and sensitive reference cultivars Shaanhe6 and Zhengyin1 respectively.

Conclusion:

Plants under water stress situation make some physiological and biochemical changes for their integrity and survival. The investigated data of current experimentation exhibited that the cultivars that have more PC and higher RWC while low MDA content are proved to be more resistant to water deficit condition compare to those having much MDA content while low RWC and PC. So, Xinong928 regarded as most resistant and Xiaoyan22 more sensitive under these indices. These results might prove to be informative in developing new drought resistant wheat cultivars with better growth and high yield in arid and semi arid zones.

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