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Original Article

Effects of drought stress factors on antibacterial activity of two *Triticum aestivum* L. varieties

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Abstract:

Triticum aestivum L. (Wheat grass), one of the members of Poaceae family, has been considered to be a very efficient therapeutic drug. In the present study, we propose to evaluate antibacterial effects of the two varieties of *T. aestivum* L. [cv. Tosunbey (drought tolerant) and cv. Sultan 95 (drought-sensitive)] which grown in three different stress conditions [(1) drought stress; (2) pre-treatment of seeds with acetyl salicylic acid; (3) drought stress and pre-treatment of seeds with acetyl salicylic acid]. The antibacterial activity of the ethanol extracts was assayed against five pathogens (*Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Escherichia coli* NRRL B-3704, *Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) by agar disc diffusion and micro broth dilution methods. The results showed that the ethanol extracts from the different studied treatments showed antibacterial activities, with the diameters of the inhibition zone ranging from 8 to 15 mm and minimum inhibitory concentration ranging from 2.5 to 20.0 µg/mL, respectively. The highest antibacterial activity, against *B. subtilis* ATCC 6633, was demonstrated by the extract of *T. aestivum* cv. Sultan 95, which grown in conditions where drought stress and pre-treatment of seeds with acetyl salicylic acid were combined.

Key words:
antibacterial activity, T. aestivum cv. Tosunbey, T. aestivum cv. Sultan 95

Apstract:

Antibiofilm aktivnost etanolnog ekstrakta *Verbascum pinnatifidum* Vahl.

Triticum aestivum L. (pšenica), jedan od članova familije Poaceae, smatra se za veoma efikasnu terapeutsku drogu. U ovom istraživanju, utvrđivan je antibakterijski efekat dva varijeteta *T. aestivum* L. [cv. Tosunbey (tolerantna na sušu) i cv. Sultan 95 (osetljiva na sušu)] koji su uzgajani u tri različita uslova stresa [(1) sres suše; (2) semena pretretirana acetil salicilnom kiselinom; (3) sres suše i pretretman semena acetil salicilnom kiselinom]. Antibakterijska aktivnost etanolnih ekstrakata ispitivana je u odnosu na pet patogenih vrsta (*Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Escherichia coli* NRRL B-3704, *Staphylococcus aureus* ATCC 25923 i *Bacillus subtilis* ATCC 6633) korišćenjem disk difuzije na agaru i mikrodilucione metode. Rezultati su pokazali da etanolni ekstrakti dobijeni iz ispitivanih različitih uslova gajenja poseduju antimikrobnu aktivnost, sa dijametrima inhibicionih zona koji su varirali od 8 to 15 mm i minimalnim inhibitornim koncentracijama od 2,5 do 20,0 µg/mL. Najveća antibakterijska aktivnost, utvrđena u odnosu na *B. subtilis* ATCC 6633, pokazana je od strane ekstrakta *T. aestivum* cv. Sultan 95, koji je rastao u uslovima kombinacije sušnog stresa i pretretmana semena acetil salicilnom kiselinom.

Ključne reči:
antibakterijska aktivnost, T. aestivum cv. Tosunbey, T. aestivum cv. Sultan 95

Introduction

Plants contain numerous of constituents and are valuable sources of new and biologically active molecules having antimicrobial properties that are important for drug designing against diseases (Das et al., 2010; Bhattacharjee and Islam, 2015; Saha et al., 2018).

Wheat as one of the members of Gramineae family, has been known for very efficient medical values and several therapeutic drugs that include bran and germ. It is used as a protection against various diseases such as constipation, heart diseases, appendicitis, obesity, diabetes and condition of the colon called diverticulum (Hadjivassiliou et al.,

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2003; Saha et al., 2018). *Triticum aestivum* has also been shown to be a rich source of Vitamins A, C, E and B complex, including B_{12} (Ashok, 2011; Sundaresan et al., 2015). It contains a multitude of minerals like calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron, and molybdenum. Other compounds that are making this grass therapeutically effective are the indole compounds, choline and laetrile (amygdalin) (Padalia et al., 2011; Sundaresan et al., 2015).

Antibiotic resistance has become a global concern (Westh et al., 2004; Saha et al., 2018) and clinical efficiency of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens as reported by Saha et al. (2018). So, there is an urgent need to develop new antimicrobial compounds which are more active against novel and re-emerging infectious diseases (Rojas et al., 1992; Saha et al., 2018). Duke and Ayensu (1985) reported that wheat is an easily grown plant in the world and that the young stems had proven effective for the treatment of biliousness, intoxication and removing skin blemishes, antipyretic, antihydrotic and sedative. *Triticum aestivum* has also been used against cough, sore throat, malaise, spasmic pain, abdominal coldness and constipation (Ashok, 2011). Wheat is also known to have anticancer and antimicrobial properties (Gregorova et al., 2015).

Drought is the most critical limiting factor for crop production and is becoming an increasingly severe problem in many regions of the world. Abiotic stress conditions such as drought, salinity, cold stress can induce the synthesis of pathogen-associated proteins (PRs) in plants (Van Loon et al., 2006; Sehgal and Mohamad, 2018). In severe drought conditions, PRs such as chitinase and glucanase have been activated in wheat (Gregorova et al., 2015). In addition, TdLTP4, a PR protein, was found to be higher in wheat variety with salt and drought tolerance (Safi et al., 2015). On the other hand, PRs induced by the effect of salicylic acid (SA) has been shown to have antimicrobial activity *in vitro* (Van Loon et al., 2006). Also, SA applications have been reported to increase antimicrobial activity in turnip (Thiruvengadam et al., 2016) and sesame plants (Hossein et al., 2016). It is known that antifungal and antimicrobial activity increases due to oxidative stress induced by environmental stresses (Sharma et al., 2018; Schmidt et al., 2019). The relationship between antimicrobial activity and PRs, produced by plants under environmental stress conditions such as defensins (Schmidt et al., 2019) and phytoalexins (Ejike et al., 2013), has been demonstrated earlier (Li et al., 2011).

In this study, the effects of drought and salicylic acid (SA) priming combinations were investigated on antibacterial activity in 21-day-old seedlings of two *T. aestivum* varieties.

Material and methods

Plant materials

The seeds were sterilized by washing with 5% sodium hypochlorite solution for 5 min (1 time) and with sterile distilled water for 2.5 min (3 times). Two tablets of 500 mg aspirin were thoroughly dissolved in 500 ml of distilled water and sterile seeds were kept in this solution for 2 hours for ascorbic acid (ASA) priming. At the end of the period, the seeds were placed on moist filter paper and germinated for three days. Seedlings were planted in perlite:peat mixture (1:3) containing pots. Plants were watered with Hoagland nutrient solution (100%; Steward, 1983) during 21 days in growth chamber (22-24 ^{o}C, photoperiod 16/8 day/night). Drought stress application was carried out with water scarcity for 5 days starting from the 21st day. Seedlings of *T. aestivum* L. cv. Tosunbey (drought tolerant) and cv. Sultan 95 (drought-sensitive) were divided into four groups: (1) control (2) drought stress; (3) pre-treatment of seeds with acetyl salicylic acid; (4) drought stress and pre-treatment of seeds with acetyl salicylic acid. Leaf sampling was done on 21-day old plants.

Preparation of plant extracts

Air-dried samples of two wheat varieties were grounded into a fine powder in a grinding mill. Pulverized plant samples (1 g) were extracted with 10 mL of 80% ethanol, (1:10 w/v) using an orbital shaker for 8 h at room temperature. The extract was separated from the solids by filtration with Whatman No. 1 filter paper. The remaining solids were extracted twice with the same solvent and extracts combined. Extracts were stored in a refrigerator (4 ^{o}C) until analyzed (Sultana et al., 2007).

Test Microorganisms

Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Escherichia coli* NRRLB-3704), Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC 6633) were used for determining the antibacterial activities of two *T. aestivum* varieties.

Screening for antimicrobial activities

Disc diffusion method was used for qualitative analyses of two *T. aestivum* species extracts (Collins et al., 1989). Studies were performed in triplicate. Treatments with Penicillin (P10) served as positive controls, respectively and treatments with ethanol without plant extracts served as negative controls.

Table 1. Disc Diffusion, MIC ratios of the two varieties of *T. aestivum* extracts

Test bacteria	Plant extracts																	
	*Disc Diffusion[a] (mm)									MIC (µg/mL)								
	cv. Tosunbey				cv. Sultan 95					cv. Tosunbey				cv. Sultan 95				
	T1	T2	T3	T4	S1	S2	S3	S4		T1	T2	T3	T4	S1	S2	S3	S4	
E.coli NRRLB-3704	8.0	11.0	10.0	7.0	12.0	11.0	7.0	7.0	16.0	20.0	2.5	10.0	20.0	2.5	2.5	20.0	20.0	4.0
P. aeruginosa ATCC 27853	10.0	9.0	7.0	7.0	11.0	7.0	9.0	9.0	8.0	5.0	5.0	20.0	20.0	2.5	20.0	10.0	2.5	1.0
P. vulgaris ATCC 13315	13.0	10.0	13.0	8.0	12.0	9.0	11.0	10.0	13.0	2.5	5.0	2.5	20.0	2.5	10.0	10.0	20.0	4.0
S. aureus ATCC 6538P	7.0	8.0	7.0	9.0	11.0	9.0	10.0	12.0	14.0	20.0	20.0	20.0	5.0	2.5	10.0	5.0	5.0	4.0
B. subtilis ATCC 6633	11.0	9.0	10.0	11.0	10.0	8.0	15.0	13.0	15.0	2.5	5.0	10.0	5.0	5.0	20.0	2.5	20.0	4.0

T: *T. aestivum* cv. Tosunbey
S: *T. aestivum* cv. Sultan 95
T1: control; T2: treatment with acetyl salicylic acid; T3: treatment with drought stress; T4: drought stress and pre-treatment of seeds with acetyl salicylic acid
S1: control; S2: treatment with acetyl salicylic acid; S3: treatment with drought stress; S4: drought stress and pre-treatment of seeds with acetyl salicylic acid
Inhibition zone (mm); a includes diameter of disk (6 mm); P10 = Penicillin (10 ug/disc); ST:Streptomycin

Minimum inhibitory concentration assay

Minimum Inhibitory Concentration (MIC) was investigated as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006). The lowest concentration of extracts inhibiting visible growth of each test microorganisms was taken as the MIC. The medium, 0.1% (w/v) Streptomycin (ST), Nystatin (NYS100) and 10% DMSO were used as the non-treated, positive and negative controls, respectively (Teanpasian et al., 2017).

Results and discussion

Tab. 1 indicated antimicrobial activity of two varieties of *T. aestivum* plant extracts and the inhibition zones formed by standard antibiotic disks and MIC ratios. The results of antimicrobial testing showed that the ethanol extracts from the different treatments studied showed variable antibacterial activities, with the diameters of the inhibition zone ranging from 8 to 15 mm and 2.5 to 20.0 µg/mL, respectively. The highest antibacterial activity was demonstrated by the extract of *T. aestivum* cv. Sultan 95, grown in drought conditions (S3 in **Tab.1**), against *B. subtilis* ATCC 6633.

Triticum aestivum plant varieties used in this study showed higher antibacterial activity against Gram-positive bacteria (*B. subtilis* ATCC 6633) than Gram-negative test bacteria. The results in present study are parallel to those reported in the previous investigations. In general, the distinctive feature of Gram-negative bacteria is the presence of a double membrane surrounding each bacterial cell. Although all bacteria have an inner cell membrane, Gram-negative bacteria have a unique outer envelope. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why gram-negative bacteria are generally more resistant to antibiotics than Gram-positive bacteria (Dülger and Dülger, 2018). In the literature there are many investigations about wheat grass antimicrobial activity (Pallavi et al., 2011; Das et al., 2012; Sundaresan et al., 2015). However, there is no data effect of drought stress on wheat grass antibacterial activity in the literature.

Drought and salinity conditions can cause oxidative stress due to osmotic stress. This may induce the emergence of PRs such as phytoalexin and defensin (Van Loon et al., 2006; Sehgal and Mohamad, 2018). Our results showed the highest antibacterial activity in drought-sensitive Sultan 95 with SA and drought treatments together. Accordingly, our results are consistent with studies in which severe drought stress activates PRs in wheat (Gregorova et al., 2015), and increases a PR which causes higher fungal resistance in drought-tolerant wheat (Safi et al., 2015). In addition, the increases in antimicrobial activity by SA priming were similarly shown to increase antimicrobial activity in SA applications in sesame (Hossein et al., 2016) and turnip (Thiruvengadam et al., 2016).

As a result, SA and drought application showed high antibacterial activity in drought-sensitive variety. This may indicate different PRs synthesized in this variety compared to the tolerant one. The occurrence of lower antimicrobial activity in the tolerant variety compared to the sensitive variety suggests that some other stress factor is needed due to its tolerance to drought.

Conclusion

This study was conducted to evaluate the antibacterial activity of two *T. aestivum* varieties under three different stress conditions. Natural products are important source of new drugs which are having importance in modern medicine. The results pointed that the ethanol extracts from plants treated with combined stress conditions had significant antibacterial activities against *P. vulgaris* ATCC 13315 (T3) and *B. subtilis* ATCC 6633 (S3).

The need to increase food production and exhaustion of wheat genetic resources has increased interest in alternative approaches for wheat improvement, including the use of different stress conditions. It is well known that abiotic stress leads to a series of morphological, physiological, biochemical, and molecular changes that adversely affect plant growth, productivity and antagonistic activity (Hayat et al., 2013; Ripa et al., 2019). Therefore, selection, screening, and application of the stress-tolerant *T. aestivum* plant varieties for better farming would considerably facilitate the farming community by overcoming such extreme climate changes.

This is the first report on the bioactivities of two varieties of *T. aestivum* grown in different drought conditions and pre-treatment of seeds with acetyl salicylic acid. This approach may also allow new kind of research in medicinal usage or development of drug research.

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References

Ashok, S.A. 2011: Phytochemical and pharmacological screening of wheat grass juice (*Triticum aestivum* L.). *International Journal of Pharmaceutical Sciences Review and Research*, 9: 159-164.

Bhattacharjee, B., Islam, S.M.S. 2015: Assessment of antibacterial and antifungal activities of the extracts of *Rhynchostylis retusa. Blume- A Medicinal Orchid. World Journal of Pharmacy and Pharmaceutical Sciences*, 4: 74-87.

CLSI, 2006: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Seventh Edition. M07- A7.

Collins, C.H., Lyne, P.M., Grange, J.M. 1989: Microbiological Methods, 6th ed. London, Butterworths, 395-410.

Das, K., Tiwari, R.K.S., Shrivastava, D.K. 2010: Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of Medicinal Plants Research*, 4: 104-111.

Das, A., Raychaudhuri, U., Chakraborty, R. 2012: Antimicrobial effect of edible plant extract on the growth of some foodborne bacteria including pathogens. *Nutrafoods,* 12: 83-88.

Duke, J.A., Ayensu, E.S. 1985: Medicinal Plants of the World. Algonac, MI (USA): Reference Publications.

Dülger, B., Dülger, G. 2018. Antibacterial activity of *Verbascum antinori. Konuralp Tıp Dergisi,* 10(3): 395-398

Ejike, C.E.C.C., Gong, M., Udenigwe, C.C. 2013: Phytoalexins from the Poaceae: Biosynthesis, function and prospects in food preservation. *Food Research International*, 52(1), 167-177.

Gregorova, Z., Kovacik, J., Klejdus, B., Maglovski, M., Kuna, R., Hauptvogel, P., Matusíkova, I. 2015: Drought-Induced Responses of Physiology, Metabolites, and PR Proteins in *Triticum aestivum*. *Journal of agricultural and Food Chemistry*, 63(37): 8125-8133.

Hadjivassiliou, M., Grunewald, R.A., Sharrack, B., Sanders, D., Lobo, A., Williamson. C. Woodroofe, N., Wood, N., Davies-Jones, A. 2003: Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. *Brain*, 126: 685-691.

Hayat, R., Khalid, R., Ehsan, M., Ahmed, I., Yokotaand, A., Ali, S. 2013: Molecular characterization of soil bacteria for improving crop yield in Pakistan. *Pakistan Journal of Botany*, 45: 1045–1055.

Hossein, S., Hosseini, S.M., Azari, A., Rafsanjani, M. H. 2018: Effects of seed priming with ABA and SA on seed germination and seedling growth of sesame (*Sesamum indicum* L.) under saline condition. *Australian Journal of Crop Science*, 12(9), 1385-1392.

Li, H., Goodwin P.H., Han, Q., Huang, L., Kang, Z. 2011: Microscopy and proteomic analysis of the non-host resistance of *Oryza sativa* to the wheat leaf rust fungus, *Puccinia triticina* f. sp. *tritici*. *Plant Cell Reports*, 31(4): 637-650.

Padalia, S., Drabu, S., Raheja, I., Gupta, A., Dhamija, M. 2010: Multitude potential of wheatgrass juice (Green Blood): An overview. *Chronicles of Young Scientists,* 1(2): 23-28.

Pallavi, K., Kumarswammy, G., Shruthi. B. 2011: Pharmacognostic investigation and antibacterial activity of *Triticum aestivum*. *Journal of Pharmacy Research*, 4: 3355-3359.

Ripa, F.A., Cao, W., Tong, S., Sun, J. 2019: Assessment of plant growth promoting and abiotic stress tolerance properties of wheat endophytic fungi. *BioMed Research International*, Article ID 6105865: 1-12.

Rojas, A., Hernandez, L., Pereda-Miranda, R., Mata, R. 1992: Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. *Journal of Ethnopharmacology*, 35: 275-83.

Safi, H., Saibi, W., Alaoui, M.M., Hmyene, A., Masmoudi, K., Hanin, M., Brini, F. 2015: A wheat lipid transfer protein (TdLTP4) promotes tolerance to abiotic and biotic stress in *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, 89, 64-75.

Saha, S., Islam, Z., Islam, S., Hossain, S., Islam, S.M.S. 2018: Evaluation of antimicrobial activity of wheat (*Triticum aestivum* L.) against four bacterial strains. *SKUAST Journal of Research,* 20(1): 58-62.

Schmidt, M., Arendt, E.K., Thery, T.L.C. 2019: Isolation and characterisation of the antifungal activity of the cowpea defensin Cp-thionin II. *Food Microbiology*, 82: 504–514.

Sehgal, O.P., Mohamad, F. 2018: Pathogenesis related proteins. In: Mandahar, C.L. (Ed.) Plant Viruses Vol. II: Pathology: 65-84. CRC Press, Boca Raton.

Sharma, A., Sharma, D., Verma, S.K. 2018: In silico study of iron, zinc and copper binding proteins of *Pseudomonas syringae* pv. *lapsa*: Emphasis on secreted metalloproteins. *Frontiers in Microbiology*, 9: 1838.

Sultana, B., Anwar, F., Przybylski, R. 2007: Antioxidant activity of phenolic components present in barks of barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. *Food Chemistry*, 104: 1106-1114.

Sundaresan, A., Selvi, A., Manonmani, H.K. 2015: The anti-microbial properties of *Triticum aestivum* (Wheat Grass). *International Journal of Biotechnology for Wellness Industries*, 4:(3): 84-91.

Van Loon, L.C., Rep, M.P. 2006: Significance of inducible defense-related proteins in infected plants. *Annual Review of Plant Pathology*, 44: 135-162.

Thiruvengadam, M., Baskar, V., Kim, S.-H., Chung, I.M. 2016: Effects of abscisic acid, jasmonic acid and salicylic acid on the content of phytochemicals and their gene expression profiles and biological activity in turnip (*Brassica rapa* ssp. *rapa*). *Plant Growth Regulation*, 80(3): 377-390.

Westh, H., Zinn, C.S., Rosdahl V.T. 2004: An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. *Microbial Drug Resistance*, 10: 169-76.