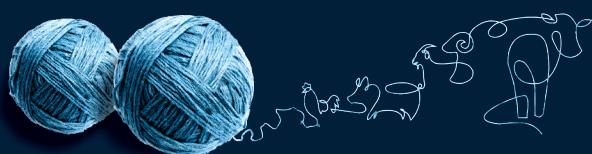


12th  
INTERNATIONAL  
SYMPOSIUM | MODERN  
TRENDS  
IN LIVESTOCK  
PRODUCTION



P R O C E E D I N G S

9 -11 October 2019, Belgrade, Serbia

**Institute for Animal Husbandry**  
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# **CONTENTS**

## **INVITED PAPERS**

<i>Čedomir Radović, Marija Gogić, Dragan Radojković, Vladimir Živković, Nenad Parunović, Aleksandar Stanojković, Radomir Savić</i> AGRO BIODIVERSITY AND LIVESTOCK FARMING: AUTOCHTHONOUS SPECIES AND BREEDS IN SERBIA (Serbia).....	1-12
<i>Vesna Gantner, Irena Jug</i> THE FUTURE OF AGRICULTURE PRODUCTION – COULD THE FORECASTED EVENTS BE ALTERED? (Croatia).....	13-22
<i>Slavča Hristov, Dušica Ostojić Andrić, Branislav Stanković</i> GENERAL PRINCIPLES AND GOOD ANIMAL WELFARE PRACTICES ON DAIRY CATTLE FARMS (Serbia).....	23-38
<i>Dušica Ostojić Andrić, Slavča Hristov, Radica Đedović, Teodora Popova, Vlada Pantelić, Dragan Nikšić, Nenad Mićić</i> EMOTIONAL STATE OF DAIRY COWS IN LOOSE AND TIED HOUSING SYSTEM - IS THERE A DIFFERENCE? (Serbia-Bulgaria).....	39-47
<i>Pero Mijić, Tina Bobić, Mirjana Baban, Maja Gregić, Franjo Poljak, Vesna Gantner</i> EFFECT OF STARTING MILK FLOW ON UDDER HEALT OF HOLSTEIN COWS (Croatia).....	48-54
<i>Dragan Nikšić, Vlada Pantelić, Dušica Ostojić Andrić, Predrag Perišić, Nenad Mićić, Marina Lazarević, Maja Petričević</i> FREQUENCY OF $\kappa$ -CASEIN AND $\beta$ -LACTOGLOBULIN GENOTYPES IN DAUGHTERS OF FIVE SIMMENTAL BULL SIRES (Serbia).....	55-63
<i>Marina I. Selionova, Magomet M. Aybazov, Milan P. Petrović, Galina T. Bobryshova, Violeta Caro Petrović</i> SCIENTIFIC DIRECTIONS OF SHEEP BREEDING DEVELOPMENT IN RUSSIA (Russia-Serbia).....	64-73
<i>Yessenbay E. Islamov, Gulzhan A. Kulmanova</i> CONDITION AND PROSPECTS OF SHEEP BREEDING DEVELOPMENT IN KAZAKHSTAN (Kazakhstan).....	74-85

<i>Violeta Caro Petrović, Milan P. Petrović Marina I. Selionova, Dragana Ružić-Muslić, Nevena Maksimović, Bogdan Cekić, Ivan Pavlović</i>	
SOME NON-GENETIC FACTORS AFFECTING LAMBS BIRTH WEIGHT IN F1 GENERATION OF MIS X ILE DE FRANCE (Serbia).....	86-93
<i>Marjeta Čandek-Potokar, Nina Batorek Lukač, Urška Tomažin, Rosa Nieto</i>	
GROWTH RATE OF LOCAL PIG BREEDS: STUDY OF PROJECT TREASURE (Slovenia-Spain).....	94-104
<i>Dubravko Škorput, Zoran Luković</i>	
SELECTION OPPORTUNITIES AND MAINTAINING GENETIC DIVERSITY IN LOCAL PIG BREEDS (Croatia).....	105-114
<i>Juan M. García Casco, Juan L. Duarte, Carmen Caraballo, Miguel A. Fernández, Patricia Palma, María Muñoz</i>	
A GENETIC EVALUATION PROGRAM FOR MEAT QUALITY TRAITS IN IBERIAN BOARS FROM DIFFERENT LIVESTOCK ORIGINS (Spain).....	115-122
<i>Patricia Palma Granados, Isabel Seiquer, Luis Lara, Ana Haro, Rosa Nieto</i>	
PROTEIN AND LIPID METABOLISM AND THEIR INTERACTION IN FATTY (IBERIAN) PIGS (Spain).....	123-136
<i>Giacomo Biagi, Monica Grandi, Carlo Pinna, Carla Giuditta Vecchiato</i>	
HOW NUTRITION MAY INFLUENCE CANINE BEHAVIOR AND COGNITIVE ABILITIES (Italy).....	137-147
<i>Aleksandar Stanojković, Čedomir Radović, Aleksandra Stanojković- Sebić, Marija Gogić, Violeta Mandić, Jakov Nišavić, Maja Petričević</i>	
ANTIMICROBIAL SUSCEPTIBILITY TESTING OF <i>STREPTOCOCCUS SUIS</i> ISOLATES TO COMMON ANTIBIOTICS USED IN PIG FARMS (Serbia).....	148-156
<i>Władysław Migdał, Bartosz Klusek, Łukasz Migdał, Anna Migdał, Maria Walczycka, Ewelina Węsierska, Marzena Zająć, Joanna Tkaczewska, Piotr Kulawik</i>	
THE CHEMICAL COMPOSITION AND QUALITY OF MEAT POLISH NATIVE CATTLE BREEDS (Poland).....	157-166

<i>Yalçın Bozkurt, Tuncay Aydogan, Cevdet Gokhan Tuzun, Cihan Dogan</i>	
A COMPUTERISED SYSTEM FOR PREDICTION OF SLAUGHTERING CHARACTERISTICS OF BEEF CATTLE (Turkey).....	167-176
<i>Maja Petričević, Dušan Živković, Dušica Ostojić Andrić, Dragan Nikšić, Veselin Petričević, Marija Gogić, Violeta Mandić</i>	
THE EFFECT OF THE FLAX SEEDS NUTRITION OF CATTLE ON PRODUCTION AND SLAUGHTER PROPERTIES (Serbia)....	177-185
<i>Giuseppe Bee, Antonia Katharina Ruckli</i>	
MORINGA OLEIFERA, AN ALTERNATIVE PROTEIN SOURCE TO SOYA IN PIG PRODUCTION? (Switzerland-Austria).....	186-190
<i>Miloš Lukić, Zdenka Škrbić, Veselin Petričević, Vesna Krnjaja, Zorica Bijelić, Nikola Delić</i>	
LAYING HENS MANAGEMENT AND NUTRITION FOR MAXIMAL EGG PRODUCTION AT 100 WEEKS OF AGE (Serbia).....	191-202
<i>Tanja Petrović, Snežana Stevanović, Dragana Paunović, Jasmina Rajić, Viktor Nedović</i>	
INNOVATION IN MEAT PACKAGING (Serbia).....	203-218
<i>Zorica Bijelić, Violeta Mandić, Vesna Krnjaja, Dragana Ružić-Muslić, Aleksandar Simić, Zdenka Škrbić, Dušica Ostojić Andrić</i>	
NITROGEN STATUS EVALUATION OF GRASS-LEGUME SWARDS UNDER FOUR N FERTILIZATION LEVELS (Serbia)	219-229
<i>Violeta Mandić, Zorica Bijelić, Vesna Krnjaja, Maja Petričević, Aleksandar Stanojković, Marija Gogić, Aleksandar Simić</i>	
SALINITY STRESS EFFECT ON SEED GERMINATION AND SEEDLING GROWTH OF SOME CROP PLANTS (Serbia).....	230-240

## **ORALLY PRESENTED PAPERS**

<i>Martin Wähner</i>	
PERSPECTIVES IN PIG FARMING IN GERMANY (Germany)..	241-249

<i>Jovan Bojkovski, Jasna Prodanov-Radulović, Milica Živkov-Baloš, Radiša Prodanović, Sreten Nedić, Sveta Arsić, Ivan Vujanac, Ivan Doborsavljević, Suzana Đedović, Renata Relić, Dušica Ostojić Andrić</i>	
BODY SCORE CONDITION OF SOWS AND THE THIN SOW SYNDROME AS HEALTH PROBLEMS ON COMMERCIAL FARMS (Serbia).....	250-258
<i>Miguel Moreno-Millán, Delia Saleno, Gabriel Anaya, Yamila Pirosanto, Florencia Azcona, Olivia Marcuzzi, Antonio Molina, Sebastián Demyda-Peyrás</i>	
A COMBINATION OF KARIOTYPING AND MOLECULAR METHODS COULD INCREASE THE DETECTION ACCURACY OF CHROMOSOMAL ABNORMALITIES IN HORSES: A CASE REPORT IN PURA RAZA ESPAÑOL HORSE (Spain-Argentina)	259-266
<i>Maha I. Hamed, Taha A. A. El-Allawy, Esraa A. Hassnein</i>	
EPIDEMIOLOGICAL AND THERAPEUTICAL STUDIES ON STRONGYLE INFECTION OF DONKEYS IN EGYPT (Egypt)..	267-284
<i>Ivan Pavlović, Snežana Ivanović, Milan P. Petrović, Violeta Caro Petrović, Dragan Ružić-Muslić, Nevena Maksimović, Bogdan Cekić</i>	
SEASON DISTRIBUTION OF GASTROTESTINAL HELMINTHS OF GOATS KEPT UNDER SEMI-INTENSIVE CONDITIONES IN NORTH WEST SERBIA (Serbia).....	285-292
<i>Antonov Valeryi Alekseevich, Grishina Marina Anatolievna, Nikolaev Sergei Ivanovich, Itskovich Aleksandr Yurievich</i>	
INCLUSION SPORE PROBIOTICS «ENSIMSPORIN» IN RATIONS OF SWINES AND ITS EFFECTS ON PRODUCTIVITY, NON-SPECIFIC AND SPECIAL RESISTANCE OF PREGNANT AND LACTATING SOWS (Russia) .....	293-304
<i>Łukasz Migdał, Krzysztof Krzysztoforski, Anna Migdał, Władysław Migdał</i>	
THE INFLUENCE OF AGE AND BREED OF PIGS ON THE CONTENT OF TOTAL AND SOLUBLE INTRAMUSCULAR COLLAGEN (Poland).....	305-315
<i>Ivan Yanchev, Kamelia Kancheva</i>	
POSIBILITIES FOR UTILIZATION OF CARBON DIOXIDE FROM POULTRY IN GREENHOUSE PLANTED LETTUCE ( <i>LACTUCA SATIVA</i> ) (Bulgaria).....	316-325

## **POSTER SECTION I**

*Marinela Enculescu*

- EVALUATION OF THE HAEMATOLOGICAL PROFILE AND SERUM ENZYMES DURING THE TRANSITION PERIOD IN DAIRY COWS (Romania)..... 326-335

*Muamer Pekmez, Admir Dokso, Muhamed Brka*

- EXTERNAL CHARACTERISTICS OF HOLSTEIN-FRIESIAN BREED ON AREA OF FEDERATION OF BOSNIA AND HERZEGOVINA (Bosnia and Herzegovina)..... 336-341

*Miloš Marinković, Predrag Perišić, Dušica Ostojić Andrić, Vlada Pantelić, Nikola Molerović, Nenad Mićić, Vladimir Živković*  
THE EFFECT OF SIRES ON THE SEMEN QUALITY OF HOLSTEIN-FRIESIAN BULLS (Serbia).....

342-351

*Ivan Čosić, Dragana Ružić Muslić, Nevena Maksimović, Bogdan Cekić, Dragan Nikšić, Nenad Mićić, Miloš Marinković*  
THE EFFECT OF PARTICULAR PARAGENETIC FACTORS ON FERTILITY AND MILK PERFORMANCE PROPERTIES OF COWS (Serbia).....

352-362

*Nenad Mićić, Miloš Marinković, Vlada Pantelić, Dragan Nikšić, Marina Lazarević, Nikola Molerović, Ivan Čosić*  
PRODUCTION PERFORMANCES AND HERD BOOK OF SIMMENTAL AND HOLSTEIN FRIESIAN CATTLE IN CENTRAL SERBIA (Serbia).....

363-372

*Madlena Andreeva, Nikola Metodiev, Bogdan Cekić, Rossen Stefanov*  
STUDY OF THE EFFECTS OF LOW TEMPERATURES ON THE MORPHOLOGICAL STATUS OF RAM SPERMATOZOA (Bulgaria-Serbia).....

373-381

*Tsonka Odjakova, Pavel Todorov, Atanaska Zgurova*  
MONITORING AND TRENDS FOR DEVELOPMENT OF SREDNORHODOPSKA SHEEP (Bulgaria).....

382-392

<i>Rossen Stefanov, Georgi Anev, Madlena Andreeva, Plamen Todorov, Nevena Maksimovic</i>	
DIFFERENT OESTRUS SYNCHRONIZATION PROTOCOLS IN LACTING NORTH-EAST BULGARIAN MERINO SHEEP IN ANESTRAL PERIOD (Bulgaria-Serbia).....	393-400
<i>Daniela Miteva, Stayka Laleva, Teodora Angelova, Daniela Yordanova, Nikolay Ivanov</i>	
QUALITY MILK COMPOSITION AND COAGULATION ABILITY IN SHEEP FROM THE BULGARIAN DAIRY SYNTHETIC POPULATION WITH DIFFERENT GENOTYPES (Bulgaria).....	401-410
<i>Jaroslava Bělková, Miroslav Rozkot, Eva Václavková</i>	
REQUIREMENTS FOR FARROWING MANAGEMENT IN HIGHLY PROLIFERATIVE SOWS (Czech Republic).....	411-422
<i>Oleksandr Tsereniuk, Oleksandr Akimov, Yuriy Chereuta, Mikola Kosov</i>	
FEATURES OF SPERM INJECTION INTO GENITAL TRACTS OF SOWS AND GILTS IN ARTIFICIAL INSEMINATION (Ukraine).....	423-430
<i>Nenad Stojiljković, Dragan Radojković, Čedomir Radović, Marija Gogić, Vladimir Živković, Radomir Savić, Aleksandar Stanojković</i>	
THE VARIABILITY OF ECONOMICALLY IMPORTANT TRAITS MONITORED IN THE PERFORMANCE TEST OF GILTS UNDER THE INFLUENCE OF FARM, YEAR AND SIRE BREED (Serbia).....	431-441
<i>Elena Cibotaru, Grigore Darie, Alisa Pirlög, Doina Plesca</i>	
THE ROLE OF ANTIOXIDANTS IN BOAR SEMEN PRESERVATION (Moldova).....	442-448
<i>Ksenija Nešić, Marija Pavlović, Vladimir Radosavljević</i>	
INSECTS – A NEW BRANCH OF ANIMAL HUSBANDRY? (Serbia).....	449-458
<i>Mirna Gavran, Dragan Dokić, Maja Gregić, Vesna Gantner</i>	
THE ASSOCIATION OF ROE DEER POPULATION WITH WEATHER CONDITIONS IN HUNTING AREA IN EASTERN CROATIA IN PERIOD 2008-2018 (Croatia).....	459-467

<i>Rositsa Shumkova, Ralitsa Balkanska</i>	
INFLUENCE OF MICROBIOLOGICAL PRODUCT BAIKAL EM1 ON THE DEVELOPMENT OF HYPOPHARYNGEAL GLANDS ON WORKER BEES AND THORACIC GLANDS ON WORKER BEES AND BEE DRONES (Bulgaria).....	468-478
<i>Dragan Dokić, Maja Gregić, Mirna Gavran, Vesna Gantner</i>	
SIGNIFICANCE OF INVESTMENTS IN AGRICULTURAL PRODUCTION ON THE EXAMPLE OF THE RURAL COUNTIES OF THE REPUBLIC OF CROATIA (Croatia).....	479-487
 <b>POSTER SECTION II</b>	
<i>Radojica Djoković, Zoran Ilić, Marko Cincović, Vladimir Kurćubić, Miloš Petrović, Milan P. Petrović, Violeta Caro Petrović</i>	
INSULIN RESISTANCE IN DAIRY COWS (Serbia).....	488-504
<i>Goran Vučković, Mirna Gavran, Maja Gregić, Pero Mijić, Ranko Gantner, Marcela Šperanda, Vesna Gantner</i>	
THE INFLUENCE OF MASTITIS RISK ON RESPONSE TO HEAT STRESS IN DAIRY SIMMENTAL COWS (Croatia).....	505-515
<i>Mahmoud R. Abd Ellah, Ghada I. Soliman, Mohamed A.H. Abd Elhakeim, Hanan K. Elsayed</i>	
EFFECT OF NATURAL <i>STRONGYLUS</i> spp. INFECTIONS ON SYNOVIAL FLUID CONSTITUENTS IN DONKEYS (Egypt)...	516-525
<i>Jasna M. Kureljušić, Aleksandra Tasić, Jadranka Žutić, Branislav Kureljušić, Ljiljana Spalević, Suzana Vidaković, Dragana Ljubojević</i>	
SURVIVAL OF SALMONELLA IN PIG CARCASSES IN SLAUGHTERHOUSES (Serbia).....	526-532
<i>Jadranka Žutić, Olivera Valčić, Branislav Kureljušić, Dobrila Jakić-Dimić, Jasna Kureljušić, Nemanja Jezdimirović, Nemanja Zdravković</i>	
SEROPREVALENCE TO <i>MYCOPLASMA HYOPNEUMONIAE</i> IN GILTS AND SOWS (Serbia).....	533-540
<i>Dragana B. Ljubojević Pelić, Suzana Vidaković, Sandra Jakšić, Miloš Pelić, Jelena Vranešević, Jasna Kureljušić, Brankica Kartalović, Milica Živkov Baloš</i>	
THE OCCURRENCE OF RESIDUE OF ANTIBIOTICS AND SULPHONAMIDES IN DIFFERENT TYPES OF HONEY (Serbia)	541-547

<i>Ivan Mičić, Zoran Rajić, Marija Mičić</i> ECONOMICS OF SUSTAINABLE AGRICULTURAL PRODUCTION AND ANALYSIS MACROINVERTEBRATES OF WATER SOURCES IN SERBIA (Bosnia and Herzegovina-Serbia).....	548-557
<b>POSTER SECTION III</b>	
<i>Bojan Stojanović, Goran Grubić, Nenad Đorđević, Aleksa Božičković, Aleksandar Simić, Vesna Davidović, Aleksandra Ivetić</i> EFFICIENCY OF PROTEIN UTILIZATION BY GRAZING RUMINANTS AND POSSIBILITY FOR IMPROVEMENT (Serbia).....	558-568
<i>Dragana Ružić-Muslić, Milan P. Petrović, Zorica Bijelić, Violeta Caro Petrović, Nevena Maksimović, Bogdan Cekić, Ivan Čosić</i> ALTERNATIVE SOURCES OF PROTEIN IN LAMB DIET (Serbia).....	569-579
<i>Vesna Krnjaja, Slavica Stanković, Ana Obradović, Tanja Petrović, Violeta Mandić, Zorica Bijelić, Marko Jauković</i> THE EFFECT OF CLIMATE CONDITIONS ON AFLATOXIN CONTAMINATION OF CEREAL GRAINS AND FEEDS (Serbia)	580-591
<i>Marija Pavlović, Aleksandra Tasić, Ksenija Nešić, Snežana Ivanović</i> SACCHAROMYCES CEREVISIAE IN FEED FOR RUMINANTS (Serbia).....	592-600
<i>Daniela Yordanova, Georgi Kalaydzhev, Stayka Laleva, Vladimir Karabashev, Teodora Angelova, Evgeni Videv</i> IN VITRO ANALYSIS OF GAS PRODUCTION OF ROUGH AND JUICY FEEDS WITH FRESH AND LYOPHILIZED RUMEN FLUID (Bulgaria).....	601-609
<i>Marzena Zająć, Joanna Tkaczewska, Piotr Kulawik, Paulina Guzik, Bronisław Borys, Władysław Migdał</i> COMPARING THE CHEMICAL COMPOSITION OF THE LAMB MEAT OF VARIOUS NATIVE BREEDS (Poland).....	610-617
<i>Vladimir Dosković, Snežana Bogosavljević-Bošković, Lidija Perić, Zdenka Škrbić, Simeon Rakonjac, Veselin Petričević</i> MEAT QUALITY OF BROILERS IN AN EXTENDED FATENING PERIOD (Serbia).....	618-624

<i>Zdenka Škrbić, Miloš Lukić, Veselin Petričević, Snežana Bogosavljević-Bošković, Simeon Rakonjac, Vladimir Dosković, Nataša Tolimir</i>	
EGG QUALITY OF COMMERCIAL LAYER HYBRID KEPT IN DIFFERENT HOUSING SYSTEMS (Serbia).....	625-632
<i>Nataša Tolimir, Marijana Maslovarić, Zdenka Škrbić, Borislav Rajković, Robert Radišić, Miloš Lukić</i>	
PREFERENCES OF CONSUMERS/CUSTOMERS FROM SERBIA TOWARD ORGANIC EGGS (Serbia).....	633-642
<i>Teodora Popova, Jivko Nakev</i>	
FATTY ACID COMPOSITION OF MUSCLE AND BACKFAT IN PIG BREEDS AND CROSSBREEDS (Bulgaria).....	643-652
<i>Vladimir Živković, Łukasz Migdał, Władysław Migdał, Ćedomir Radović, Marija Gogić, Slavča Hristov, Nenad Stojiljković</i>	
INFLUENCE OF SIRE BREED ON MEATINESS OF PIG CARCASS (Serbia-Poland).....	653-658
<i>Milica Živkov Baloš, Sandra Jakšić, Nenad Popov, Suzana Vidaković, Dragana Ljubojević Pelić, Jasna Prodanov Radulović, Željko Mihaljev</i>	
ELECTRICAL CONDUCTIVITY OF DIFFERENT TYPES OF THE SERBIAN HONEY (Serbia).....	659-665
<i>Aleksandra M. Tasić, Tijana D. Mitrović, Marija Pavlović, Jasna Kureljušić</i>	
A COMPARISON OF TWO METHODS FOR DETERMINATION OF HMF IN HONEY: HPLC METHOD VERSUS SPECTROPHOTOMETRIC METHOD (Serbia).....	666-673
<i>Jordan Marković, Tanja Vasić, Dragan Terzić, Dragoslav Đokić, Jasmina Milenković, Mladen Prijović, Đorđe Lazarević</i>	
CARBOHYDRATE AND PROTEIN FRACTIONS, AND FERMENTATION CHARACTERISTICS OF COMMON VETCH – OAT SILAGES (Serbia).....	674-683
<i>Vesna Dragičević, Milena Simić, Branka Kresović, Milan Brankov</i>	
HOW CROPPING SYSTEMS AFFECT PHOTOSYNTHETIC PIGMENTS AND MAIZE GRAIN YIELD (Serbia).....	684-694

<i>Milena Milenković, Milena Simić, Milan Brankov, Vesna Perić, Miodrag Tolimir, Vesna Dragičević</i>	
COMPETITIVE ABILITY OF SOYBEAN AND PROSO MILLET IN DIFFERENT INTERCROP COMBINATIONS (Serbia).....	695-703
<i>Tanja Vasić, Snežana Andjelković, Jordan Marković, Sanja Živković, Đorđe Lazarević, Mladen Prijović</i>	
MYCOPOPULATION OF DIFFERENT FABA BEAN GENOTYPES IN SERBIA (Serbia).....	704-711

## SALINITY STRESS EFFECT ON SEED GERMINATION AND SEEDLING GROWTH OF SOME CROP PLANTS

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Invited paper

**Abstract:** Soil salinity is one of the most important factors limiting crop productivity. It is known that the agricultural soils with high concentrations of salts increase day by day. For this reason, it is imperative to monitor the tolerance of crops and genotypes to salt stress because they have different threshold sensitivity. Generally, higher salinity levels in the media negatively affect the germination of seeds and seedling growth of most crops. Germination, germination speed and seedling growth parameters significantly decrease with increasing salt concentrations in the media. Soil salinity adversely affects germination, resulting in poor plant stand. Therefore, the development of salt tolerant genotypes of crops with promising yields would be an ideal solution for growing plants on these soils, as well as sustainable food and feed production.

**Key words:** germination, seedling growth, osmotic stress, crop

### Introduction

Soil is the most limiting factor for crop production in the worldwide. Therefore, the production of food for humans and feed for animals is limited. The soil salinization is big problem for agricultural productivity worldwide. Generally, crop plants are sensitive to soil salinity. Soil salinity is the abiotic factor that limits the germination and early seedling growth of most crop plants. *Jamil et al. (2011)* estimate that more than 50% of the arable land will be salinized because of low rainfall, high surface evaporation, weathering of native rocks, irrigation with saline water and poor agricultural practices, by 2050. In Serbia, about 4.6% of agricultural soils are saline and alkaline soils. Saline soils are contaminated with 40 mM NaCl salts and have  $EC_e > 4 \text{ dS m}^{-1}$  and osmotic potential  $< 0.117 \text{ MPa}$  (*Ashraf, 2009*). High levels of soil salinity reduce the ability of plants to absorb water and plant growth (*Munns, 2002*), impair ions absorption (*Karimi et al.,*

2005), increase accumulation of a toxic ions (*Nawaz et al., 2010*). The salinity negatively affects seed germination, seedling growth and enzyme activity (*Seckin et al., 2009*), growth, development, yield and quality of plants (*Jouyban, 2012*). *Shahbaz and Ashraf (2013)* conclude that soil salinity reduces cultivated land area, productivity and quality of crops. *Ashraf (2004)* points that the saline soil causes osmotic stress due to low osmotic potential of soil solution, ionic stress because specific toxicity effect on ions, nutritional imbalances or a combination of mentioned factors. Also, *Muscolo et al. (2013)* has proved that the high soil salinity has a strong impact on plants because it causes osmotic stress, oxidative stress, ion toxicity, nutritional disorders, alteration of metabolic processes, membrane disorganization and reduction of cell division and expansion. *Mandić et al. (2011)* conclude that testing of genotypes at early seedling growth would be especially helpful in identification and selection of genotypes for particular soil. In this paper, we characterize the effect of the induced water deficit by NaCl (osmotic stress) on germination and seedling growth of a few crops commonly sowed in areas of Serbia. Testing of genotypes of crops at the early seedling stage under different concentrations of NaCl in the growing medium could be helpful in the identification and selection of genotypes for cultivation on saline soils.

## **Effect of NaCl-induced osmotic stress on seed germination and seedling growth of maize**

The high-quality seeds are essential for successful crop production. However, water availability and movement in the medium are important factors which promote germination, root and shoot elongation. The germination is not possible under conditions of low water potential (*Singh et al., 2013*). Minimum moisture in the soil is necessary for germination of seeds, i.e. for the restart of embryonic axis growth, the intensification of breathing and other metabolic activities and releasing of energy and nutrients (*Carvalho and Nakagawa, 1988*). However, high salt content of the soil causes lower osmotic potential in the soil solution than in the seed cells which prevents the absorption of water. Therefore, the key to problem is osmoregulation in the first phase of salt stress. Na toxicity affects the seedling growth in the second phase of salt stress (*Schubert et al., 2009*). The soil limits germination and early seedling growth, crop growth and productivity (*Flowers, 2004*).

Maize is moderately sensitive to salinity (*Ouda et al., 2008*), but the maize genotypes differ in resistance and tolerance to salt (*Khodarahmpour, 2012*). The maize hybrids vary in their tolerance and phytotoxicity to high soil salinity. Thus, *Mandić et al. (2014a)* have concluded that Serbian maize hybrids cannot tolerate high salt concentrations in the medium, where the hybrid ZP 666 showed better

tolerance than hybrid ZP 560 (Table 1 and 2). These authors find that the germination energy, germination, root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight of maize seedling decrease significantly with increasing osmotic stress induced by NaCl. Authors have concluded that the germination sensitivity threshold of maize is treatment with -0.3 MPa. The osmotic stress causes loss of turgidity of the cells involved in elongation which inhibits the growth of root.

If salinity levels in the soil increases, seed germination of maize decreases (*Khayatnezhad and Gholamin, 2011; Miroslavljević et al., 2013*). Generally, under salinity conditions, the root elongation is more sensitive than shoot elongation (*Demir and Arif, 2003*). *Leishman and Westoby (1994)* find that maize genotypes with longer root systems have higher resistance to low osmotic stress. Salt stress reduces germination and parameters of maize seedlings due to ion toxicity, osmotic and oxidative stress (*Sozharajan and Natarajan, 2014*). Generally, Na<sup>+</sup> and Cl<sup>-</sup> are metabolically toxic to cell (*Taiz and Zeiger, 2002*). Many researches have showed that increased salt concentration in the medium reduces germination rate, germination speed, root and shoot length, germination index, root and shoot dry weight of maize seedling and seedling vigor index (*Carpici et al., 2009; Idikut, 2013*).

**Table 1.** The effects of hybrid and different osmotic stress on germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW).

Factor	GE %	G %	RL cm	ShL cm	RFW mg	ShFW mg	RDW mg	ShDW mg
Hybrid (A)								
ZP 560	16.9	63.2	4.2 <sup>b</sup>	1.1 <sup>b</sup>	45.8	32.5 <sup>b</sup>	6.5 <sup>b</sup>	4.2 <sup>b</sup>
ZP 666	15.8	65.3	6.4 <sup>a</sup>	2.1 <sup>a</sup>	45.9	43.9 <sup>a</sup>	7.6 <sup>a</sup>	6.0 <sup>a</sup>
F test	ns	ns	**	**	ns	**	*	**
Osmotic stress, MPa (B)								
0	34.9 <sup>a</sup>	97.2 <sup>a</sup>	12.2 <sup>a</sup>	4.7 <sup>a</sup>	82.3 <sup>a</sup>	135.6 <sup>a</sup>	10.7 <sup>a</sup>	12.5 <sup>a</sup>
-0.3	30.5 <sup>ab</sup>	94.0 <sup>ab</sup>	7.8 <sup>b</sup>	2.0 <sup>b</sup>	61.8 <sup>b</sup>	48.9 <sup>b</sup>	8.9 <sup>b</sup>	6.9 <sup>b</sup>
-0.6	24.8 <sup>b</sup>	83.0 <sup>b</sup>	5.2 <sup>c</sup>	1.8 <sup>b</sup>	48.3 <sup>c</sup>	15.3 <sup>c</sup>	7.9 <sup>bc</sup>	3.9 <sup>c</sup>
-0.9	6.2 <sup>c</sup>	60.8 <sup>c</sup>	3.1 <sup>d</sup>	0.6 <sup>c</sup>	39.4 <sup>c</sup>	13.3 <sup>c</sup>	6.8 <sup>c</sup>	3.0 <sup>cd</sup>
-1.2	1.2 <sup>d</sup>	37.8 <sup>d</sup>	2.0 <sup>e</sup>	0.4 <sup>c</sup>	26.4 <sup>d</sup>	10.9 <sup>c</sup>	4.9 <sup>d</sup>	2.6 <sup>d</sup>
-1.5	0.2 <sup>e</sup>	13.0 <sup>e</sup>	1.3 <sup>e</sup>	0.2 <sup>c</sup>	16.9 <sup>d</sup>	5.1 <sup>c</sup>	3.2 <sup>e</sup>	1.5 <sup>e</sup>
F test	**	**	**	**	**	**	**	**
A × B	ns	ns	**	**	ns	**	ns	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; \*\* - significant at 1% level of probability, \* - significant at 5% level of probability and ns - not significant

**Table 2. The effects of hybrid and different osmotic stress on rate germination index (RGI), seedling vigor index (SVI), relative seedling water content (RSWC), phytotoxicity of root (PhR), phytotoxicity of shoot (PhSh) and dry matter stress tolerance index (DMSI).**

Factor	RGI %	SVI	RSWC %	PhR %	PhSh %	DMSI %
Hybrid (A)						
ZP 560	2.7	457.6 <sup>b</sup>	83.8 <sup>a</sup>	55.4	69.8 <sup>a</sup>	48.9 <sup>b</sup>
ZP 666	3.3	706.5 <sup>a</sup>	81.2 <sup>b</sup>	57.6	62.5 <sup>b</sup>	55.9 <sup>a</sup>
F test	ns	**	*	ns	**	**
Osmotic stress, MPa (B)						
0	10.3 <sup>a</sup>	1642.2 <sup>a</sup>	89.2 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	100.0 <sup>a</sup>
-0.3	4.0 <sup>b</sup>	920.4 <sup>b</sup>	85.5 <sup>a</sup>	35.4 <sup>b</sup>	57.6 <sup>b</sup>	68.3 <sup>b</sup>
-0.6	2.2 <sup>bc</sup>	583.9 <sup>c</sup>	81.3 <sup>b</sup>	56.8 <sup>c</sup>	65.0 <sup>b</sup>	51.0 <sup>c</sup>
-0.9	0.8 <sup>c</sup>	223.5 <sup>d</sup>	80.9 <sup>b</sup>	74.0 <sup>d</sup>	88.3 <sup>c</sup>	42.1 <sup>d</sup>
-1.2	0.8 <sup>c</sup>	97.9 <sup>de</sup>	79.2 <sup>b</sup>	83.6 <sup>e</sup>	91.1 <sup>c</sup>	32.4 <sup>e</sup>
-1.5	0 <sup>c</sup>	24.4 <sup>e</sup>	78.9 <sup>b</sup>	89.4 <sup>f</sup>	94.8 <sup>c</sup>	20.3 <sup>f</sup>
F test	**	**	**	**	**	**
A × B	ns	**	*	ns	**	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; \*\* - significant at 1% level of probability, \* - significant at 5% level of probability and ns - not significant

## Effect of NaCl-induced osmotic stress on seed germination and seedling growth of red clover

The legumes, especially red clover, are highly salt-sensitive. *Asci (2011)* concludes that the germination and seedling growth of legume on saline soils determines the degree of crop establishment. Salinity resistance to germination seeds of legume is heritable trait important for the selection of salt-resistant genotypes (*Ashraf et al., 1987*). In general, high salt concentration in the germination medium negatively affects the germination and seedling growth of most legumes, such as Egyptian, red and Persian clovers (*Gravandi, 2013*), strawberry clover (*Can et al., 2013*), white clover and alfalfa (*Zhanwu et al., 2011*), *Medicago rutherfordica* (*Guan et al., 2009*) and yellow sweet clover (*Ghaderi-Far et al., 2010*). *Mandić et al. (2014b)* have reported that the Serbian red clover cultivars (Kolubara, K-32, K-17 and K-39) are very sensitive to salt, especially cv. K-32 (Table 3). Authors report that germination, seedling growth and vigor index significantly decrease with increasing salt concentration in the germination medium. In general, salinity lowers osmotic potential, resulting in decreased

availability of water. Increasing salinity levels significantly decrease vigor index, and hence the ability of a seed to produce normal seedlings.

In another study, *Mandić et al. (2019)* have found that the cv. K-17 has higher germination energy, germination and vigor index than cv. K-32 and that the tested parameters have not differed between 0 and 50 mM NaCl. However, increases in salt concentration over 100 mM NaCl significantly reduce these parameters.

**Table 3. The effects of cultivar and NaCl concentration level on germination energy (GE), germination (G), percentage of dead or infected seeds (DIS), percentage of hard seed (HS), normal (NS) and abnormal seedlings (AS), root length (RL), shoot length (ShL), fresh (FW) and dry weight of seedling (DW) and seedling vigor index (SVI)**

Factor	GE %	G %	DIS %	HS %	NS %	AS %	RL cm	ShL cm	FW g	DW g	SVI
<b>Cultivar (A)</b>											
Kolubara	53.6 <sup>a</sup>	63.6 <sup>a</sup>	36.2 <sup>b</sup>	0.2 <sup>c</sup>	52.4 <sup>a</sup>	11.2	1.5 <sup>b</sup>	4.8	9.15	1.12	472.9 <sup>b</sup>
K-32	24.4 <sup>c</sup>	34.3 <sup>c</sup>	60.7 <sup>a</sup>	5.0 <sup>b</sup>	21.7 <sup>c</sup>	12.6	1.7 <sup>ab</sup>	5.2	10.42	1.44	285.8 <sup>d</sup>
K-17	49.2 <sup>a</sup>	62.6 <sup>a</sup>	35.2 <sup>b</sup>	2.2 <sup>c</sup>	51.6 <sup>a</sup>	11.0	1.9 <sup>a</sup>	5.4	9.80	1.33	530.6 <sup>a</sup>
K-39	34.3 <sup>b</sup>	46.6 <sup>b</sup>	24.4 <sup>c</sup>	29.0 <sup>a</sup>	38.2 <sup>b</sup>	8.4	1.8 <sup>a</sup>	5.0	9.38	1.22	388.0 <sup>c</sup>
F test	***	***	***	**	**	ns	*	ns	ns	ns	**
<b>NaCl concentration effects mM NaCl (B)</b>											
0	86.1 <sup>a</sup>	88.6 <sup>a</sup>	3.6 <sup>a</sup>	7.8	78.1 <sup>a</sup>	10.5	2.4 <sup>a</sup>	7.6 <sup>a</sup>	16.27 <sup>a</sup>	1.71 <sup>a</sup>	888.6 <sup>a</sup>
50	60.0 <sup>b</sup>	69.8 <sup>b</sup>	21.0 <sup>b</sup>	9.2	58.8 <sup>b</sup>	11.0	2.3 <sup>ab</sup>	6.9 <sup>b</sup>	15.36 <sup>a</sup>	1.59 <sup>a</sup>	638.7 <sup>b</sup>
100	36.5 <sup>c</sup>	52.0 <sup>c</sup>	38.8 <sup>c</sup>	9.2	40.8 <sup>c</sup>	11.2	2.0 <sup>b</sup>	5.4 <sup>c</sup>	9.80 <sup>b</sup>	1.34 <sup>ab</sup>	382.3 <sup>c</sup>
150	14.2 <sup>d</sup>	30.8 <sup>d</sup>	60.0 <sup>d</sup>	9.2	21.0 <sup>d</sup>	9.8	1.3 <sup>c</sup>	3.4 <sup>d</sup>	5.61 <sup>c</sup>	1.12 <sup>b</sup>	136.1 <sup>d</sup>
200	5.0 <sup>e</sup>	17.8 <sup>e</sup>	72.2 <sup>e</sup>	10.0	6.2 <sup>e</sup>	11.5	0.7 <sup>d</sup>	2.1 <sup>e</sup>	1.39 <sup>d</sup>	0.61 <sup>c</sup>	50.7 <sup>e</sup>
F test	***	***	***	ns	**	ns	**	**	**	**	**
A × B	**	**	**	ns	**	ns	ns	ns	ns	ns	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; \*\* - significant at 1% level of probability, \* - significant at 5% level of probability and ns - not significant

*Tavakkoli et al. (2010)* point out that the high concentrations of NaCl reduce seedling growth due to increased concentration of Na<sup>+</sup> and Cl<sup>-</sup>. For this reason, the seedling growth is reduced (length of root and shoot and fresh and dry weight of root and shoot). Red clover seedlings at the medium with high salt content have lower accumulation of dry matter due to which the seedlings are short and small weight.

## **Effect of NaCl-induced osmotic stress on seed germination and seedling growth of field pea**

Field pea genotypes differ in salinity tolerance at seedling stage. They can be classified into three groups: sensitive, intermediate and resistant to salinity. Previous research shows that Serbian cultivars of field pea are tolerant to salt stress during germination and early embryo growth (*Jovičić et al., 2010; Petrović et al. 2016*). These authors conclude that the germination and seedling quantitative parameters of field pea significantly decrease with increasing NaCl in the medium. The seedling grows slowly under high levels of salinity due to a slow water uptake by seeds, ions are involved in the physiological processes and damage the cell. Thus, *Mer et al. (2000)* report that the Na<sup>+</sup> in the large amounts negatively affects cell division, metabolism and imbalance of other nutrients. *Mandić et al. (2016)* have found genetic variability between field pea cultivars Kosmaj and Letin for germination, seedling parameters, seedling vigor index, relative seedling water content and phytotoxicity of root (Table 4 and 5). According to their results, cv. Kosmaj has significantly higher germination energy, shoot length, shoot fresh and dry weight, relative seedling water content and phytotoxicity of root, while lower germination, root length, root fresh and dry weight and seedling vigor index than cv. Letin. Also, they have found that the all investigated parameters, except phytotoxicity of root and shoot, significantly decrease with increasing osmotic stress. Phytotoxicity of root and shoot significantly increase with increasing osmotic stress.

**Table 4. The effects of cultivar and osmotic stress on germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW).**

Factor	GE %	G %	RL cm	ShL cm	RFW mg	ShFW mg	RDW mg	ShDW mg
Cultivar (A)								
Kosmaj	55.4 <sup>a</sup>	92.1 <sup>b</sup>	5.6 <sup>b</sup>	5.6 <sup>a</sup>	49.6 <sup>b</sup>	79.3 <sup>a</sup>	6.3 <sup>b</sup>	7.9 <sup>a</sup>
Letin	27.8 <sup>b</sup>	98.6 <sup>a</sup>	10.3 <sup>a</sup>	2.2 <sup>b</sup>	90.2 <sup>a</sup>	57.5 <sup>b</sup>	13.2 <sup>a</sup>	6.7 <sup>b</sup>
F test	**	**	**	**	**	**	**	*
Osmotic stress, MPa (B)								
0	89.2 <sup>a</sup>	98.9 <sup>a</sup>	11.2 <sup>a</sup>	9.0 <sup>a</sup>	100.2 <sup>a</sup>	136.9 <sup>a</sup>	13.6 <sup>a</sup>	13.6 <sup>a</sup>
-0.3	70.4 <sup>a</sup>	98.1 <sup>a</sup>	9.6 <sup>b</sup>	5.4 <sup>b</sup>	93.1 <sup>a</sup>	112.9 <sup>b</sup>	13.2 <sup>a</sup>	11.4 <sup>b</sup>
-0.6	45.6 <sup>b</sup>	98.0 <sup>a</sup>	9.2 <sup>b</sup>	3.6 <sup>c</sup>	75.7 <sup>b</sup>	71.3 <sup>c</sup>	10.8 <sup>b</sup>	8.1 <sup>c</sup>
-0.9	37.5 <sup>b</sup>	96.8 <sup>a</sup>	7.4 <sup>c</sup>	3.3 <sup>c</sup>	63.6 <sup>c</sup>	54.6 <sup>c</sup>	7.2 <sup>c</sup>	6.0 <sup>d</sup>
-1.2	5.6 <sup>c</sup>	92.9 <sup>b</sup>	5.3 <sup>d</sup>	1.5 <sup>d</sup>	44.6 <sup>d</sup>	23.8 <sup>d</sup>	7.0 <sup>c</sup>	3.1 <sup>e</sup>
-1.5	1.2 <sup>c</sup>	87.5 <sup>c</sup>	5.0 <sup>d</sup>	0.7 <sup>d</sup>	42.1 <sup>d</sup>	10.9 <sup>d</sup>	6.8 <sup>c</sup>	1.6 <sup>e</sup>
F test	**	**	**	**	**	**	**	**
A × B	**	**	**	**	*	ns	**	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; \*\* - significant at 1% level of probability, \* - significant at 5% level of probability and ns - not significant

**Table 5. The effects of cultivar and osmotic stress on seedling vigor index (SVI), relative seedling water content (RSWC), phytotoxicity of root (PhR), phytotoxicity of shoot (PhSh) and dry matter stress tolerance index (DMSI) of field pea**

Factor	SVI	RSWC %	PhR %	PhSh %	DMSI %
Cultivar (A)					
Kosmaj	1068.1 <sup>b</sup>	87.8 <sup>a</sup>	44.6 <sup>a</sup>	58.0	61.7
Letin	238.0 <sup>a</sup>	86.1 <sup>b</sup>	15.3 <sup>b</sup>	51.7	63.8
F test	**	**	**	ns	ns
Osmotic stress, MPa (B)					
0	1991.8 <sup>a</sup>	88.6 <sup>a</sup>	0 <sup>a</sup>	0 <sup>a</sup>	100.0 <sup>a</sup>
-0.3	1474.8 <sup>b</sup>	88.0 <sup>a</sup>	15.7 <sup>b</sup>	29.8 <sup>b</sup>	90.4 <sup>b</sup>
-0.6	1256.4 <sup>c</sup>	87.2 <sup>a</sup>	18.2 <sup>c</sup>	55.5 <sup>c</sup>	68.9 <sup>c</sup>
-0.9	1036.1 <sup>d</sup>	88.8 <sup>a</sup>	34.7 <sup>c</sup>	66.3 <sup>c</sup>	50.4 <sup>d</sup>
-1.2	637.5 <sup>e</sup>	85.4 <sup>b</sup>	54.1 <sup>d</sup>	85.6 <sup>d</sup>	37.1 <sup>e</sup>
-1.5	521.6 <sup>e</sup>	83.7 <sup>b</sup>	57.0 <sup>d</sup>	91.9 <sup>d</sup>	30.0 <sup>e</sup>
F test	**	**	**	**	**
A × B	**	*	**	*	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; \*\* - significant at 1% level of probability, \* - significant at 5% level of probability and ns - not significant

## Conclusions

Salt stress causes huge losses in crop production worldwide. The high salt concentration in the solutions had effect on germination and seedling parameters in the crops studied. Significant differences were found in germination of seeds and seedling parameters between genotypes of crops. The choice of crops and genotypes can be a way for their cultivation on salinity soil environments in a sustainable and productive way. It is therefore necessary to start with identification and selection of varieties for cultivation on saline soils during the seedling stage.

## Uticaj sonog stresa na klijanje i rast klijanaca nekih useva

Violeta Mandić, Zorica Bijelić, Vesna Krnjaja, Maja Petričević, Aleksandar Stanojković, Marija Gogić, Aleksandar Simić

## Rezime

Zaslanjivanje zemljišta je jedan od najvažnijih faktora koji ograničava produktivnost useva. Poznato je da površina poljoprivrednog zemljišta sa visokom

konzentracijom soli raste iz dana u dan. Iz tog razloga, neophodno je pratiti toleranciju useva i genotipova na stres soli jer imaju različitu graničnu osetljivost. Generalno, viši nivoi saliniteta u medijuma negativno utiču na klijavost semena i rast klijanaca većine useva. Klijavost, brzina klijanja i rast klijanaca značajno se smanjuju sa povećanjem koncentracije soli u medijumu. U suštini, salinitet i kiselost negativno utiču na kljanje, što dovodi do lošeg (proređenog) sklopa. Stoga bi razvoj genotipova tolerantnih na soli sa obećavajućim prinosima bilo idealno rešenje za gajenje biljaka na takvim zemljištima, kao i za održivu proizvodnju hrane.

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