

## IMPROVING SALT TOLERANCE IN *TRIFOLIUM ALEXANDRINUM* L. THROUGH INTERSPECIFIC HYBRIDIZATION, POLYPLOIDIZATION AND INDUCED VARIATIONS

K. DWIVEDI<sup>1</sup>, A.K. ROY, P. KAUSHAL<sup>2</sup>, S. PATHAK, D.R. MALAVIYA<sup>3\*</sup>

ICAR – Indian Grassland and Fodder Research Institute, Jhansi-284003, India

<sup>1</sup> Present Address Amity University Madhya Pradesh, Gwalior-474005, India

<sup>2</sup> Present Address ICAR-National Institute of Biotic Stress Management, Raipur-493225, India

<sup>3</sup> Present Address ICAR-Indian Institute of Sugarcane Research, Lucknow-226002, India

E-mail: drmalaviya47@rediffmail.com

*Soil salinity significantly affects crop productivity throughout the world. Improving intrinsic salt tolerance of the plants may effectively improve productivity. In vitro evaluation is an effective and quick method allowing utilization of inter and intra genotypic variation in a controlled environment. Trifolium alexandrinum is one of the most important winter season annual fodder crop in India and Mediterranean region. Diverse T. alexandrinum genotypes were evaluated in vitro for salt tolerance. Intra and inter genotypic variability was observed for response to varying levels of salt stress at different growth stages. Germination was adversely affected with increasing salt stress among genotypes, however, three genotypes EC 318954, ISH 34/41, ISH 34/8Y showed 75–80 % germination even at 0.75 % salt level. High seedling mortality was observed at higher salinity levels except EC 318954 which showed low mortality at 0.50 and 0.75 % salinity. Seedlings with normal root growth ranged from 5 to 80 % at 0.25 and 0.5 % salinity. Based on average Salinity Susceptibility Index (SSI) the ISH progenies were most tolerant (SSI = 0.895) for germination as well as radicle and plumule length, number of leaves and plant weight (SSI = 0.91). ISH progenies, tetraploids, Fahli ecotype and multifoliolate showed better tolerance. The study confirmed successful transfer of salinity tolerance from T. apertum to T. alexandrinum. The petiole and hypocotyl explants at moderate salinity and petiole explants at high salinity responded well for in vitro callusing. Calli developed at 0.75 % salinity can be a source of developing tolerant lines through natural cell line selection. Embryo culture response of Mescavi genotypes was better than Fahli and Saidi genotypes.*

© K. DWIVEDI, A.K. ROY, P. KAUSHAL, S. PATHAK, D.R. MALAVIYA, 2022

**Key words:** Egyptian clover, ecotypes, tetraploid, interspecific cross, multifoliolate.

## ПОКРАЩЕННЯ СОЛЕСТІЙКОСТІ *TRIFOLIUM ALEXANDRINUM* L. ЗА ДОПОМОГОЮ МІЖВИДОВОГО СХРЕЩУВАННЯ, ПОЛІПЛОЇДИЗАЦІЇ ТА ІНДУКОВАНОЇ МІНЛИВОСТІ

Засолення ґрунтів має суттєвий вплив на продуктивність рослин у всьому світі. Вдосконалення природної солестійкості рослин може ефективно покращити продуктивність. *In vitro* оцінювання — це ефективний і швидкий метод, який дозволяє використовувати генотипну мінливість між видами та всередині виду у контрольованому середовищі. *Trifolium alexandrinum* є однією з найважливіших озимих однорічних кормових рослин в Індії та Середземноморському регіоні. Різноманітні генотипи *T. alexandrinum* оцінювали на предмет солестійкості *in vitro*. Генотипну мінливість між видами та всередині виду спостерігали для визначення реакції на різні рівні сольового стресу на різних етапах росту. Підвищення рівня сольового стресу між генотипами мало негативний вплив на проростання насіння, однак, три генотипи EC 318954, ISH 34/41, ISH 34/8Y продемонстрували 75–80 % проростання насіння навіть при рівні солі в 0,75 %. При вищих рівнях засолення спостерігали високий рівень загибелі саджанців, окрім EC 318954, який продемонстрував низький рівень загибелі при рівнях засолення в 0,50 та 0,75 %. Саджанці з нормальним ростом коренів були у діапазоні від 5 до 80 % при рівні засолення 0,25 та 0,5 %. За розрахунками середнього індексу сприйнятливості до засолення (SSI), потомство ISH продемонструвало найвищий рівень стійкості (SSI = 0,895) щодо проростання насіння, а також довжини зародкового корінця і зародкової бруньки, кількості листочків і ваги рослини (SSI = 0,91). Потомство ISH, тетраплоїди, екотип Fahli та багатолісткові продемонстрували вищу стійкість. Дослідження підтвердило успішне перенесення солестійкості від *T. apertum* до *T. alexandrinum*. Експланти живців і гіпокотіля при помірному засоленні та експланти живців при високому рівні засолення позитивно відреагували на калюсогенез *in vitro*. Калюси, які розвинулись при засоленні в 0,75 %, можуть бути джерелом розробки стійких ліній за допомогою природної селекції клітинних ліній. Реакція ембріональної культури генотипів Mescavi була кращою, ніж генотипів Fahli і Saidi.

**Ключові слова:** єгипетська конюшина, екоטיפи, тетраплоїд, міжвидове схрещення, багатолісткові.

REFERENCES

- Abogadallah GM (2010) Sensitivity of *Trifolium alexandrinum* L. to salt stress is related to the lack of long-term stress-induced gene expression. *Plant Science* 178:491–500. doi:10.1016/j.plantsci.2010.03.008
- Al-Ansari EM (2003) Salinity tolerance during germination in two arid-land varieties of wheat (*Triticum aestivum* L.). *Seed Science and Technology* 31:597–603
- Al-Khatib MM, Mc Neilly T, Collins JC (1994) Between and within cultivars variability in salt tolerance in Lucerne (*Medicago sativa* L.). *Genet Res Crop Evol* 41:156–164
- Ashraf M, Orooj A (2006) Salt stress effects on growth, ion accumulation and seed oil content in an arid zone medicinal plant ajwain (*Trachyspermum ammi* L.). *Sprague J Arid Environ* 64:209–220. 10.1016/j.jaridenv.2005.04.015
- Ashraf M, Mc Meilly T, Bradshaw AD (1986) The response to NaCl and ionic content of selected salt-tolerant and normal lines of three legume forage species in sand culture. *New Phytologist* 104:463–471
- Ballhorn DJ, Elias JD (2014) Salinity-mediated cyanogenesis in white clover (*Trifolium repens*) affects trophic interactions. *Annals of Botany* 114:357–366
- Barakat MN, Abdel Latif TH (1996) *In vitro* selection of wheat callus tolerant to high levels of salts and plants regeneration. *Euphytica* 91:127–140
- Bayuelo-Jimenez JS, Debouck DG, Lynch JP (2002) Salinity tolerance in *Phaseolus* species during Early Vegetative Growth. *Crop Science* 42:2184–2192
- Burgutin AB, Butenko RG, Kaurov BA, Iddegodia N (1996) *In vitro* selection of potato for tolerance to sodium chloride. *Russian Journal of Plant Physiology* 43:524–531
- Chaudhary MT, Wainwright ST, Merett MJ (1996) Comparative NaCl tolerance of Lucerne plants regenerated from salt selected suspension cultures. *Plant Sci* 114:221–232
- Dehnavi AR, Zahedi M, Ludwiczak A, Perez SC, Piernik A (2020) Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy* 10:859. doi:10.3390/agronomy10060859
- Duncan RR, Wakson RM, Nabors MW (1995) *In vitro* screening and field evaluation of tissue cultured regenerated sorghum (*Sorghum bicolor* L.). *Euphytica* 85:373–380
- Fisher RA, Maurer R (1978) Drought resistance in spring wheat cultivars. I. Grain yield response. *Austr J Agric Res* 29:897–912
- Foolad MR, Jones RA (1993) Mapping salt tolerance genes in tomato (*Lycopersicon esculentum*) using trait-based marker analysis. *Theor App Genet* 87:184–192
- Ghassemabadi HF, Eisvand HR, Akbarpour OA (2018) Evaluation of salinity tolerance of different clover species at germination and seedling stages. *Iran J Plant Physiol* 8(3):2469–2477
- Hasegawa PM, Bressan RA, Nelson DE, Samaras Y, Rhodes Y (1995) Tissue culture in the improvement of salt tolerance in plants. In: Yeo AR, Flowers TJ (eds) *Soil Mineral Stresses: Approaches to Crop Improvement*. pp. 83
- Huoying C, Jianhua Z, Xiaoning Z (2002) *In vitro* selection of NaCl tolerance mutant of *Lycopersicon esculentum* Mill. *J Shanghai Agric Coll* 20:1–6.
- Isayenkov SV (2012) Physiological and molecular aspects of salt stress in plants. *Cytology and Genetics* 46:302–318. doi: 10.3103/S0095452712050040
- Isayenkov SV (2019) Genetic sources for the development of salt tolerance in crops. *Plant Growth Regulation* 89(1):1–17. <https://doi.org/10.1007/s10725-019-00519-w>
- Isayenkov SV and Maathuis FJM (2019) Plant Salinity Stress: Many Unanswered Questions Remain. *Frontiers in Plant Science*. 10:80.doi: 10.3389/fpls.2019.00080
- Kaushal P, Malaviya DR, Mahanta SK, Roy AK (2003) Nutritive value of diploid and improved tetraploid lines of Egyptian clover (*Trifolium alexandrinum*) at different cutting stages. *Ind J Anim Sci* 73(8):940–944.
- Kaushal P, Malaviya DR, Roy AK, Kumar B, Tiwari A (2005) *Trifolium alexandrinum* × *T. resupinatum* - interspecific hybrids developed through embryo rescue. *Plant Cell, Tissue and Organ Culture* 83:137–144
- Kaur A, Kaur KP, Kalia A, Rani U, Kahlon JG, Sharma R, Malaviya D, Kapoor R, Sandhu JS (2017) Generation of interspecific hybrids between *Trifolium vesiculosum* and *T. alexandrinum* using embryo rescue. *Euphytica* 213:253. doi: 10.1007/s10681-017-2042-x
- Kazemeini SA, Pirasteh-Anosheh H, Basirat A, Akram NA (2018) Salinity tolerance threshold of berseem clover (*Trifolium alexandrinum*) at different growth stages. *Pakist J Bot* 50(5):1675–1680.
- Kintzios SE, Barberaki M, Aivalakis G, Drossopoulos J, Holevas CD (1997) *In vitro* morphogenetic responses of mature wheat embryos to different NaCl concentrations and growth regulator treatments. *Plant Breeding* 116:113–118
- Mandal AK, Reddy GPO, Ravisankar T (2011) Digital database of salt affected soils in India using Geographic Information System. *J Soil Salin Water Quality* 3(1):16–29
- Malaviya DR, Roy AK, Kaushal P, Kumar B, Tiwari A (2004) Development and characterization of interspecific hybrids of *Trifolium alexandrinum* × *T. apertum* using embryo rescue. *Plant Breeding* 123:536–542
- Malaviya DR, Kumar B, Roy AK, Kaushal P, Tiwari A (2005) Estimation of variability for isozymes of five enzyme systems among wild and cultivated species of *Trifolium*. *Genet Res Crop Evol* 52:967–976
- Malaviya DR, Roy AK, Kaushal P, Chakraborti M, Yadav A, Khare A, Dhir R, Khairnar D, George GP (2018) Interspecific compatibility barriers, development

- of interspecific hybrids through embryo rescue and lineage of *Trifolium alexandrinum* (Egyptian clover) – important tropical forage legume. *Plant Breeding* 137:655–672. doi: 10.1111/pbr.12616
- Malaviya DR, Raman H, Dear BS., Raman R, Roy AK, Kaushal P, Chandra A, Hughes SJ (2019) Genetic Diversity and Lineage Based on SSR Markers of Two Genomic Resources among *Trifolium* Collections Held within the Australian Pastures Genebank. *Open J Genet* 9:1–14
- Malaviya DR, Roy AK, Kaushal P, Yadav A, Pandey DK (2019) Complementary gene interaction and xenia effect controls the seed coat colour in interspecific cross between *Trifolium alexandrinum* and *T. apertum*. *Genetica* 147(2):197–203
- Malaviya DR, Roy AK, Anand A, Choubey RN, Baig MJ, Dwivedi K, Kushwaha N, Kaushal P (2019) Salinity tolerance of *Panicum maximum* genotypes for germination and seedling growth. *Range Management & Agroforestry* 40(2):227–235
- Malaviya DR, Roy AK, Kaushal P, Pathak S, Kalendar R (2021) Phenotype study of multifoliolate leaf formation in *Trifolium alexandrinum* L. *PeerJ* 9:e10874 <http://doi.org/10.7717/peerj.10874>
- Mirza JI, Tariq R (1993) The growth and nodulation of *Trifolium alexandrinum* as affected by salinity. *Biol Plant* 35(2):289–292
- Moore G (1998) ‘Soilguide: A handbook for understanding and managing agricultural soils’, Bulletin 4343, Department of Agriculture, Western Australia, Perth.
- Munns R (2002) Comparative physiology of salt and water stress. *Plant Cell Environment* 25:239–250
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15:473–497
- Pakar N, Pirasteh-Anosheh H, Emam Y, Pessaraki M (2016) Barley growth, yield, antioxidant enzymes, and ion accumulation affected by PGRs under salinity stress conditions. *J Plant Nutrition* 39:1372–1379
- Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. *Ecotoxicol Environ Safety* 60:324–349
- Pathak S, Malaviya DR, Roy AK, Dwivedi K, Kaushal P (2015) Multifoliolate leaf formation in induced tetraploids of *Trifolium alexandrinum* L. *Cytologia* 80(1):59–66
- Philips GC, Collins GB (1984) Red clovers and other forage legumes. In Sharp WR, Evans DA, Ammirato PV and Yamada Y (eds.) *Handbook of plant cell culture*. Mcmillan Publ Co. New York. 2:169–210
- Pirasteh-Anosheh H, Emam Y, Sepaskhah AR (2015) Improving barley performance by proper foliar applied salicylic-acid under saline conditions. *Inter J Plant Produc* 9(3):467–486.
- Rogers ME, Nobel CL (1990) Breeding for increased salt tolerance in irrigated pasture legume. In: Management of soil salinity in South East Australia. Humphrey E, Murihead WA, Lelji A (eds) *Proceedings of a symposium held at Albury, NSW, Australia*. Austral Soc Soil Scie 191–200 p
- Roy AK, Malaviya DR, Kaushal P (1998) Production potential of induced tetraploid lines in comparison to diploid lines of *Trifolium alexandrinum*. *Forage Research* 24(1):7–11
- Roy AK, Malaviya DR, Kaushal P, Kumar B, Tiwari A (2004) Interspecific hybridization of *T. alexandrinum* with *T. constantinopolitanum* using embryo rescue. *Plant Cell Reports* 22:605–610.
- Roy AK, Malaviya DR, Kaushal P (2005) Pollination behaviour among different breeding populations of Egyptian clover. *Plant Breeding* 124:171–175
- Roy AK, Malaviya DR, Kaushal P (2016) Genetic improvement of fodder legumes especially dual purpose pulses. *Ind J Genet Plant Breed* 76(4):608–625
- Roy AK, Malaviya DR, Anand A, Choubey, Baig MJ, Dwivedi K and Kaushal P. 2021. Salinity tolerance of *Avena sativa* fodder genotypes. *Tropical Grasslands-Forrajés Tropicales* 9(1):109–119.
- Shakur AB, Kay NA, Stevens DP, Skibinski DOF (1988) Salt tolerance in natural populations of *T. repens*. *New Phytologist* 109:483–490
- Verma P, Chandra A, Roy AK, Malaviya DR, Kaushal P, Pandey D, Bhatia S (2015) Development, characterization and cross-species transferability of genomic SSR markers in Berseem (*Trifolium alexandrinum* L.), an important multi-cut annual forage legume. *Molecular Breeding* 35:23
- Winicov I (1996) Characterization of rice (*Oryza sativa*) plants regenerated from salt tolerant cell lines. *Plant Sciences* 113:105–111
- Winter E, Lauchli A (1982) Salt tolerance of *Trifolium alexandrinum* L. Comparison of salt response of *T. alexandrinum* and *T. Pratense*. *Austr J Plant Physiol* 9:221–226
- Zhao R, Gao S, Qiao Y, Zhu H, Bi Y (1995) Studies on the application of anther culture in salt tolerance breeding in wheat (*Triticum aestivum*). *Acta Agronom Sinica* 21:230–234
- Zhu JK (2000) Genetic analysis of plant salt tolerance using Arabidopsis. *Plant Physiology* 124:941–948. doi: 10.1104/pp.124.3.941
- Zouhaier B, Mariem M, Mokded R, Rouached A, Alsane K, Chedly A, Abderrazek S, Abdallah A (2016) Physiological and biochemical responses of the forage legume *Trifolium alexandrinum* to different saline conditions and nitrogen levels. *J Plant Res* 129(3):423–434. doi: 10.1007/s10265-016-0791-6.

Received November 27, 2020  
 Received April 28, 2021  
 Accepted May 18, 2022