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THE EFFECT OF COLD AND 2-HNA (2 HYDROXY-NICOTINIC ACID) TREATMENTS ON ISOLATED MICROSPORE CULTURE AND ANDROGENIC RESPONSE OF DIFFERENT BRAZILIAN WHEAT GENOTYPES

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Microspores are gametic cells with an extraordinary capacity of giving rise into a new plant via in vitro embryogenesis. Doubled haploids generated by isolated microspore culture are completely homozygous, and represent an important tool for research in plant genetics and breeding. Stress treatments act as a trigger and are employed to switch microspores from gametophytic development to a sporophytic pathway. The most utilized stress treatments in cereals are cold, heat and starvation. With the aim to determine the best type of stress treatment and to evaluate in vitro culture response, five different Brazilian wheat genotypes were tested, and two stress treatments were applied: cold (4 ?C) and a combination of heat (32 ?C) and 2-hydroxynicotinic acid (2-HNA, 100 mg/L). Wheat cultivar Pavon 76 was used as androgenic positive control. Microspore purification was carried out using ten spikes per genotype. Spikes were sampled when microspores were in the early to mid uninucleate stages. Microspores were purified by gradient centrifugation. With the exception of the positive control (showing no differences between treatments), our results demonstrated that cold treatment was the most efficient type of stress, producing green plants in four out five tested genotypes. Only two genotypes treated with the combination of heat and 2-HNA produced plants, and one of them produced one single albino plant. One genotype did not produce green plants, just albinos, in both stress treatments. Albino plants are the bottleneck for the production of doubled haploids by androgenesis, and to minimize their occurrence is a great challenge. Although albinism is a major genetic characteristic, also influenced by environmental conditions, some level could be avoided with appropriated stress treatment and culture medium optimization. In this study, a Brazilian wheat genotype was identified as highly androgenic, showing significant genotype interaction. In addition to the production of doubled haploids for breeding or for genetic studies, the present results may be interesting for transgenic purposes. Microspores are considered promising targets for genetic transformation. As an immediate product of meiosis, the microspore represents the haploid chromosome set, where the transgene will be inserted. With embryogenic competence and genome duplication, the desired trait is fixed in T0 homozygous plants. Testing a protocol using microspore for transformation is our next step.