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Ethyl methanesulfonate (EMS)-Induced Mutagenesis of Durum Wheat for TILLING

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[Introduction]

Mutation breeding is one of the popular and effective tools for crop improvement. There are many published procedures for the mutagenesis of wheat using chemical mutagen such as ethyl methanesulphonate (EMS), but there is no consistency in terms of the optimal conditions for effective EMS mutagenesis. In view of this, the aim of the present study was two-fold: First, to model and optimize the factors influencing the germination and survival rate of EMS-induced durum wheat mutants. Second, to investigate phenotypic variation of M_1 and M_2 mutant generations.

[Materials and Methods]

Response surface methodology (RSM) was used to model and optimize EMS conditions. The independent variables were EMS concentration (% v/v), EMS exposure time, and imbibition time (hours); while response variables were germination (%) and survival rate (%). Based on the optimized EMS mutagenesis conditions, wild type seeds (M_0) were treated with 0.7% EMS solution for 18 hours. Sodium thiosulphate (100 mM) was used to inactivate the effect of the EMS prior to seeding. The wild type and mutant seeds were planted in Chonbuk National University experimental site in 2015. In the subsequent growing season, the M_1 generation plants were self-fertilized to produce the M_2 generation. More than 4,000 M_2 plants were generated from M_1seeds. Similarly, the M_2 seeds were also planted again in a plant-to-row fashion to produce the next (M_3) generation. The presence of mutant phenotypes was examined throughout thewholegrowthstageinallgenerations.

[Results and Discussions]

Prior to the optimization experiment, the optimal EMS concentration was determined by taking different concentration of EMS solutions (0.30 to 0.90) holding the imbibition (5 hours) and treatment time (18 hours) at a constant using dose response curve. The curve predicted the suitable dose of the EMS needed to achieve at least the 50 % germination to be at 0.75 %. However, this method does not take into account the interaction of the different factors involved at different time series. Hence, another method that aimed at determining the best concentration of EMS solution, imbibition time and EMS exposure time combinations by employing RSM on the basis of central composite design (CCD) was used. The germination rate of plants with EMS dose 0.75 % using dose response curve was closest to the targeted percent of germination with 0.70 % EMS dose by employing RSM. Thus, the 0.70% EMS treatment was chosen for the development of the TILLING population. The optimal mutagenesis conditions for 50 to 60 % germination rate obtained from response surface analysis were: EMS concentration 0. 7 %, EMS treatment time 18 h, imbibition time 4 h.

Phenotypic characteristics of M_1 and M_2 mutant population were recorded.126 plants out of the total 1,215M_1 plants exhibited abnormal phenotypic characteristic. Only 430 plants out of 1,215 M1 plants produced fertile M_2 seeds. In M_1 generations, 46 out of the126 mutant phenotypes were chimeral leaf mutants. Mutant phenotypes were observed in less than 3 % of the M_2 mutant population. Generally, the altered phenotypes were observed in the leaf structure, leaf pigmentation, seed morphology and developmental characteristic such as maturity time. Higher altered mutant phenotypes were recorded in the area of pigmentation followed by morphological phenotypes, and then followed by developmental features. The application of our EMS mutagenesis protocol may be helpful to produce experimental population suitable for TILLING and ecoTILLING experiment in durum wheat while minimizing deleterious effects on viability and fertility.

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