Abstra ct The oc currence of f boron tox xicity becom mes a majo or constrain nt in cereal l production n in the world, thus cause significant t yield loss particularl y in wheat , one of th he most sus sceptible crops to o boron tox xicity.List of ei ghty Aegilo ops-wheat a accessions c collected fro om differen nt country a and used analysis of f the present t study Name of f The Species * *Genome Country Aegilops speltoides S S Aegilops Aegilops Aegilops Aegilops Aegilops Aegilops longissima sharonensis bicornis searsii kotschyi S1 S Ssh S Sb S Ss S US U cylindrostach hys U US Aegilops euvariabilis US U Aegilops geniculata sy yn. ovata U UM Name of f the accession ns TS-100, TS-02, TS-41 1, TS-43, Israel (7) TS-47, T TS-76, TS-01 Unknown (1 ) TS-117 Turkey (1) TS-118 Syria (1) TS-132 TL-01, T TL-02, TL-04 4, TL-05, Israel (8) TL-07, T TL-09, TL-17 7, TL-21 Jordan (1) TL-24 TH-17, T TH-02, TH-03 3, TH-04, TH -07, Israel (9) TH-10, T TH-11, TH-15 5, TH-01 Israel (2) TB-04, T TB-05 Egypt (3) TB-07, T TB-10, TB-12 2 Israel (4) TE-03, T TE-09, TE-21 , TE-27 Syria (1) TE-36 TKK-01 , TKK-03, TK KK-06, Israel (6) Tkk-11, TKK-27, TKK KK-21 Israel (4) TKC-01 , TKC-04, TK KC-06, TKC-0 08 Unknown (1 ) TKE-02 TKE-03 , TKE-12, TKE-19, Israel (9) TKE-24 , TKE-42 , TKE-46, TK KE-62, TKE-6 66, Unknown (1 ) TO-01 Israel (2) TO-07, T TO-13 TKE-22, 44 www.macroth hink.org/jab Journal of A J Applied Biote echnology ISSN 2 2327-0640 2015, Vol.Few primers s were take en from w wheat chrom mosomes, 5 5A, 5B, an nd 5D bec cause recen nt study (Schnur rbusch et al l., 2008) ide entified som me orthologo ous sequenc ce of B tole erant genes on these chromo osomes. The e total PCR reaction vo olume was 1 13 ul, comp posed of 2.0 0 ul genomi ic DNA, 1.5 ul 1 10X PCR bu uffer (Tris w with 15 mM M MgCl2, Co onc. 10x), 0 0.75 ul dNT TPs (Contain ns dCTP, dGTP, d dTTP and dATP all in n the conc. 2.2 Hyd droponic scr reening The hyd droponic sc creening wa as done in a a growth ch hamber with h light set to o 16 h day at 22 oC and 8 h h night at 15 oC. Hig gh quality s seeds were sterilized i in a solutio on of 1.3% sodium hypoch lorite (2.5% % bleach sol lution) plus s one drop o of Tween 20 0 (surfactan nt) in distille ed water 43 www.macroth hink.org/jab Journal of A J Applied Biote echnology ISSN 2 2327-0640 2015, Vol.Agrono omic manag gement of B B toxicity is more of se erious task t than dealing g with B de eficiency (Sutton et al., 200 07). Boron s stress is on ne of the ke ey limiting factors for crop produ uction in 42 www.macroth hink.org/jab Journal of A J Applied Biote echnology ISSN 2 2327-0640 2015, Vol. 3, No. 2 many ar reas of the world (Jeff feries et al., 2000). Mate erials and M Methods 2.1 Plan ls nt Materials Seventy y-nine geno otypes from m 12 wild species w within the A Aegilops-Tri iticum grou up were obtaine d from Dr. Moshe Feld dman, Depa artment of P Plant Scienc ces, The We eizmann Ins stitute of Science e, Rehovot 7 76100, Isra el. These g enotypes w were collecte ed from sev veral Medite erranean region s sources (Ta able 1).2.3 DNA NA extraction n Fresh le eaves from 13-days ol ld seedlings s were used d for DNA extraction f followed by y CTAB mini-pr rep method (IRRI, 199 97). The D DNA sampl es were an nalyzed both h qualitativ vely and quantita atively usin g a spectrop photometer and 0.8% a agarose gel electrophor resis. 2.4 SSR R marker ge notyping Thirty-f four selecte ed SSR prim mers (along with three Barley prim mers) were used for th he study.Sample es were subj jected to the e following g thermal pr rofile for am mplification n in a therm mocycler: after the e initial 7 m min at 95 ?C C, SSR mark ker amplific cation comp prised 10-15 5 touchdow wn cycles of 94 ?C C for 30 s, a annealing fo for 30 s, dec creasing the temperatur re by 0.5 ?C C per cycle u until the specifie ed annealing g temperatu ure was

reac ched, and 7 72 ?C for 30 0s.T The first m matrix was p produced fro om the stan ndardized ro oot length d data using S SIMINT procedu ure based o on Average Taxonomic c Distance (i.e. DIST coefficient t in the pro ocedure). Standar rdization of f data was d done by STA TAND proce edure of NT TSYS. The second ma atrix was generat ed with hel p of SSR da ata using N Nei genetic d distance (Ne ei, 1972).Previou us studies w were inappro opriate in co ontext of the e number of f species inc cluded and the type of mole ecular mark ker used fo or the diver rsity studies s. On the o other hand w wild emme er wheat Triticum m dicoccoi ides and ry ye (Secale e cereale) are also i important g genetic sto ocks for improve ement of cu ultivated wh heat.Ae. speltoid des, Ae euv variablis an d Ae. sears sii also exp perience tole erance perf formance ag gainst B toxicity y. Due to t the inconsi stency, no reliable co onclusion c can be draw wn for any y of the genotyp pes from the e species Ae e. kotschyi a and Ae. bico ornis. Wheat is very suscept ible to B to oxicity and p production is hampere d in toxic e environment ts. Due to th he stock of diffe erent genes in an inte erbreeding p population used in br reeding pro ograms, the genetic diversit ty among w wheat cultiva ars has decr reased (We ei et al., 200 00, as cited in Li et al. ., 2006). M Moreover, mo ost of the pr revious stud dies (Konsta antinos et a al., 2010; Be elkadi et al., 201 1, Baghizad deh et al., 2 2011, Nagha avi et al., 2 2009) that w were devoted d to identif fying the genetic relationsh ip and div versity of A Aegilops sp pecies wer re based on n RAPD m markers.2.5 Stat tistical Anal lysis The alle ele size at e each microsa atellite locu us was meas sured in bas se pairs by u using Alpha aEaseFC 45 www.macroth hink.org/jab Journal of A J Applied Biote echnology ISSN 2 2327-0640 2015, Vol.Deve elopment of f tolerant va ariety is no w of utmos st importan nce since agronom mic manage ement of so oil boron is b becoming i ineffective t to mitigate t the toxicity y. Due to narrow genetic bas se of the ex xisting whe eat cultivars s, genetic v ariation for r this charac cter was poorly reported.Their der ivatives gen notypes TL L-09 and TL L-17 were found to ex xplained conside erable tolera ance to boro on. On other r hand, amo ong the SSR R markers us sed, Xgwm 192 was the mos st robust in n identifying g boron tol lerance poly ymorphism.B But, surpris singly these species gav ve better root len ngth under 3 3 mM B. Am mong the sp pecies evalu uated Ae. sh haronensis, Ae. longiss sima and Ae. kots schyi reveal led better to olerance ag gainst exces s B than th e other spec cies (Figure e 1). Resu ults 3.1 Mor rphological l Characteri ization 3.1.1 Ro oot Length Variation In the h hydroponic screening, variation in n root lengt th of the ge notypes of each specie es under differen nt concentra ations (0, 3 and 10 mM M) is presen nted in the Figure 1.3, No. 2 for 8 m minutes and then thorou ughly rinsed d. Seeds we ere placed o on filter pap per containin ng 0.2% of Pipra acil (Pipera acillin Sodiu um antibiot tic) solution n in labeled d petri dishe es for germ mination.The Aegilops g genera are s still neglect ted in B toleranc ce research, , and the ex xistence of s such a large e pool of ge enotypic var riation is pr romising and can n be used in n wheat bre eding progr rams for de eveloping B -tolerant cu ultivars.3.69 (Felsenstei in, 2009) w ith 1000 permuta ation and co o-phenetic c correlation c coefficient w was calcula ated by NTS SYSpc prog gram ver. 2.11 (R Rohlf, 2005 5) for inter rnal validat tion of the phylogene etic tree.?C until electrop phoresis...3.46