



Supplemental Figure 1. Lack of yeast, two-hybrid, interaction between HaHSFA9 and HaDREB2. Representative results for assays performed with the Hf7c strain transformed with different combinations of plasmids. Lane 1: pGAD424 + pGBT9 (empty vectors). Lane 2: pGAD424 + pGBT9:HaHSFA9 (3WA). The pGBT9:HaHSFA9 (3WA) plasmid contains a mutant version of HaHSFA9, in which the three tryptophan residues of the three putative the AHA motifs (Almoguera *et al.*, 2002) were substituted for alanine. The mutant HaHSFA9 (3WA) was transcriptionally inactive in yeast (compare with lane 5). Lane 3: pGBT9 + pGAD424:HaDREB2. Lane 4: pGBT9:HaHSFA9(3WA) + pGAD424:HaDREB2. Lane 5: pGAD424 + pGBT9:HaHSFA9 (wild type factor). A positive control for two-hybrid interaction is shown in lane 6: pGAD424:2H8.3 + pGBT9:HaHSFA9 (3WA). 2H8.3 is a cDNA from sunflower embryos cloned by two-hybrid interaction with HaHSFA9. Aliquots of each yeast strain were diluted and spotted on the different media (as indicated in the legend of Fig. 4) then incubated for 3 days at 30°C. Similar results were obtained with different transformations for each plasmid combination, or after assaying growth for 5-6 days at 25°C (data not shown, lower temperature conditions used for detection of weak, protein-protein, interaction). In one single experiment, we could detect a very weak interaction between HaHSFA9(W3A) and HaDREB2. However, this result was not confirmed by assays using a different yeast strain, PJ69-49.