

APPENDIX VIII - RESPONSE TO PRE-APPLICATION REVIEW

In a maximum of 2 pages, provide responses to the reviewer comments and recommendations provided to you in your Pre-Application Summary of Review.

If these were addressed in your Full Application, please indicate specifically where in the application changes were made. If you disagreed with the reviewers' comments, please explain your rationale for why no changes were necessary.

We felt that the reviewer's comments were constructive and have modified the proposal according to their suggestions as outlined below:

1. The main criticism was that our proposal was "too comprehensive for the resources and proposed timeline."

Response: We agree that the proposal was/is very ambitious and have made several changes to reduce overall workload. These include (1) a reduction in the size of the transcriptome analysis (**Activity 1.5**) from 960 samples to 576 samples; and (2) a reduction in the number of species and genotypes for the population genomic analyses from 4 to 3 and 2,000 to 1,500, respectively (**Activity 2**). In addition, we will phenotype and evaluate the female MAGIC population only (this reduces the number of RILs from 1,200 to 600), and only in year 3 (**Activity 3**), so that it will be possible to complete data analyses and manuscript submission by the project end date. Lastly, we have increased the size of the requested budget by circa 20% to increase the resources available to conduct the project.

2. A second concern was whether or not the 288-line population contained sufficient variation and was large enough, with sufficient replicates, to have resolving power to identify the determinants of these complex stress tolerances.

Response: This is a legitimate concern. Fortunately, preliminary analyses of the SAM population have revealed significant genetic associations for a variety of traits relevant to the present proposal, including salt tolerance (measured as Geometric Mean Productivity growth of reproductive biomass across high & low salt), as well as leaf $\delta^{13}\text{C}$, leaf N, and leaf mass per area measured under optimal conditions (reported in **B. Study System**). However, we do worry about power in the field screens due to greater environmental variation. Therefore, we have increased the number of replicates in the field screens from three to four (**Activity 1.1**), despite the greater workload. In addition, we are collaborating with the software company, SAP, to develop genome-wide association mapping algorithms that can incorporate genotype x environment interactions (**Activity 1.2**). This should allow us to combine greenhouse and field results for a given stress into a single model, thereby increasing power.

3. The sequencing of 2000 genotypes and associated soil characteristics is also ambitious, but the expected outcomes of these studies are not clear.

Response: As mentioned above, we have reduced the number of genotypes from 2,000 to 1,500. We agree that the anticipated outcomes of this work were not fully articulated in the pre-proposal. We view the population genomic data and association/QTL mapping data from the SAM and MAGIC populations to be highly complementary and necessary to meet the goals of the proposed research (see discussion in **Activity 2**). Because of low LD in the natural populations, we should be able to identify the specific gene (and site in some instances) that is

targeted by selection. On the other hand, the phenotypic effects of this variation may be unclear. With the association mapping/QTL data, we will have confidence in the phenotypic effects of a given locus, but it may contain multiple genes and SNPs.

In addition, the results from the population genomic analyses will allow us to address fundamental biological questions (see discussion in **Activity 2**), including the extent of overlap in genes under selection for various stresses, the extent of gene re-use during adaptation in the different species, the types of genes under selection (regulatory vs. structural), and the positions of these genes within regulatory networks.

4. The incorporation of the GE³LS information into the project should be clarified, as should the methodology to be used. The scope of the GE³LS questions beyond the MTA issue could also be extended. The likely influence of the white paper on MTAs for germplasm was questionable.

Response: We now discuss the GE³LS methods in detail (**Activities 6.1 and 6.2**) and describe how the GE³LS team will provide internal reports and advice to the project team (rather than a white paper) on how to reduce barriers to uptake of innovative germplasm (**VIII EXPECTED SOCIAL AND/OR ECONOMIC BENEFITS: C. Plan for knowledge translation and development of benefits**). We also extend the scope of the GE³LS questions to the more general issue of how agricultural data will be shared in the future (**Activity 6.2**). Lastly, we have added a wholly new GE³LS activity (**Activity 5**), which focuses on the development of crop yield models that will enable predictions of likely yields of new stress resistant sunflower cultivars (and competitor crops) in different soil and climate conditions across Canada.