Practice abstract #2.6

Fast and effective sample collection for cross-pollinating species and their genome wide genotyping.

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CHALLENGE

The cross-pollinating species like Buckwheat or Lupin cannot by genotyped as pure lines since each cultivar is mixture of lines with different level of heterozygosity. To capture genome variability (molecular marker polymorphisms) needed for mapping of loci associated with important agronomical traits two methods called the Genome-Wide Allele Frequency Fingerprints (GWAFFs) or DArTseq can be used. To provide sufficient insight into the variability in each cultivar, about hundred individuals from each cultivar have to be sampled as small leaf discs for DNA extraction. In the field or greenhouse, this is a laborious and space and time consuming task.

SOLLUTION

The use of young seedling can tackle the problem. The prerequisite is that the seed lot of each variety tested is large enough to provide at least one hundred seedlings for DNA extraction and enough seed for all phenotyping experiments. Therefore, one hundred and fifty seeds of each variety are sown for DNA

extraction on wet paper in a Petri dish with a diameter of 20 cm. In comparison, such number of seedlings sown in field or greenhouse would require about one and a half square meter. The petri dish with the seeds is placed to dark at room temperature in a climate cabinet. When the seedlings' roots reach about 2-3 cm, a 2 cm root segment is taken from hundred seedlings, and DNA is extracted from the whole batch.

OUTCOME

We found that this approach is independent on outdoor conditions and much faster. It also allows organization of the task independently on growing stage of the plant (all samples are taken at the same growing stage). The extracted DNA is much purer compared the DNA extracted from leaves, because of lack of presence of secondary metabolites and proteins associated with the photosynthesis and free of DNA from matured chloroplasts.

About CROPDIVA

CROPDIVA wants to put 6 underused arable crops back in the fields:
oats, hull-less barley for human consumption, triticale, buckwheat, faba beans and lupins.
27 European partners are joining forces to enhance agrobiodiversity in Europe.
They will achieve this by focusing on crop diversity and creating local value chains.

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