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Published in:
Plant Breeding

Link to article, DOI:
10.1111/pbr.12445

Publication date:
2017

Document Version
Peer reviewed version

1-24-2017

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Abstract
The use of doubled haploids (DHs) in maize has become ubiquitous in maize breeding programmes as it allows breeders to go from cross to evaluation in as little as 2 years. Two important aspects of the *in vivo* DH system used in maize are as follows: (i) the identification of haploid progeny and (ii) doubling of the haploid genome to produce fertile inbred lines. This study is focused on the first step. Currently, identification of maize haploid progeny is performed manually using the *R1-nj* seed colour marker. This is a labour-intensive and time-consuming process; a method for automated sorting of haploids would increase the efficiency of DH line development. In this study, six inbred lines were crossed with the maternal haploid inducer 'RWS/RWK-76' and a sample of seed was sorted manually for each line. Using the VideometerLab 3 system, spectral imaging techniques were applied to discriminate between haploids and hybrids. Using DNA markers to confirm the haploid/diploid state of the tested seed, for the majority of genotypes haploid identification was possible with over 50% accuracy.

Disciplines
Agronomy and Crop Sciences | Plant Breeding and Genetics

Comments
This is an accepted manuscript of an article published as De La Fuente, Gerald N., Jens Michael Carstensen, and Michael A. Edberg. "Discrimination of haploid and diploid maize kernels via multispectral imaging." *Plant Breeding* 136, no. 1 (2017): 50-60. doi:10.1111/pbr.12445/full.

Discrimination of Haploid and Diploid Maize Kernels via Multispectral Imaging

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Abstract

The use of doubled haploids (DHs) in maize has become ubiquitous in maize breeding programs as it allows breeders to go from cross to evaluation in as little as two years. Two important aspects of the in vivo DH system used in maize are: 1) the identification of haploid progeny and 2) doubling of the haploid genome to produce fertile inbred lines. This study is focused on the first step. Currently identification of maize haploid progeny is done manually using the *R1-nj* seed color marker. This is a labor intensive and time consuming process, a method for automated sorting of haploids would increase the efficiency of DH line development. In this study, six inbred lines were crossed with the maternal haploid inducer 'RWS/RWK-76' and a sample of seed was sorted manually for each line. Using the VideometerLab 3 system, spectral imaging techniques were applied to discriminate between haploids and hybrids. Using DNA markers to confirm the haploid/diploid state of the tested seed, for the majority of genotypes haploid identification was possible with over 50% accuracy.

Keywords maize, haploid, diploid, fluorescence, automated sorting

Introduction

In the world of plant breeding, speed is key, as expressed in the genetic gain equation (De la Fuente et al., 2013). Per cycle gains are limited by the denominator which generally contains time and cost. Over time, breeders found various ways to accelerate the timeline by using tools such as winter nurseries and early generation testing. However, it still takes time to generate the final inbred line to a level of homozygosity and homogeneity which is acceptable. Rapid development of 100% homozygous and homogeneous lines is accomplished by development of doubled haploid (DH) lines (Geiger, 2009).

DHs are used with great success in other crops besides maize (*Zea mays* L.), and their use and acceptance continues to increase in maize breeding. Development of DH lines is more demanding compared to inbred line development by continued self-pollination. First discovered in the 1940s by Chase (1949), haploid plants in maize are naturally occurring at a low frequency. Their utility for genetics and breeding was recognized, but use of DHs was not immediately accepted due to the low frequency of haploid kernels and inability to efficiently produce fertile haploid plants. Subsequent development of the maternal haploid inducer 'Stock 6' (Coe, 1959) and other improved inducer lines, and development of economic and applicable protocols for the production of DH lines led to a dramatic increase in line development using the *in vivo* maternal haploid system in maize during the past two decades (Geiger, 2009).

For successful *in vivo* maternal haploid induction, a few key steps must be met. First, haploids must be generated on the maternal donor plant. Second, kernels with haploid embryo

("haploids") must be distinguishable and separated from undesirable hybrid kernels. Third, haploid plants are treated with colchicine to double their genome number and self-pollinated to generate the final DH line. Herein, we focus on the second step: successful identification of haploids out of a mixture with undesirable hybrid kernels. On average, we expect that approximately 10% of the total number of kernels in a given lot of induced kernels will be haploid. As a consequence, 90% of the kernels are undesirable as their embryo contains 50% each donor and inducer genomes. Although alternative selectable markers are under investigation, the most widely and successfully used selectable marker is *R1-nj* (personal communication with various breeding companies). *R1-nj*, is successful due to its dominant inheritance, and ability to distinguish between its transmission to the triploid endosperm and the diploid embryo. *R1-nj* produces a red coloration in the cap of the aleurone (endosperm transmission) and in the embryo (embryo transmission). By observing this coloration, it is possible to distinguish haploids (color in the cap of the aleurone, but none in the embryo) reliably from hybrids (color in the cap of the aleurone and in the embryo). Although several other dominant inherited phenotypic markers exist in maize, *R1-nj* has so far been superior due to xenia expression and ability to select at the seed level before planting. Thus, only haploid kernels are colchicine treated and planted, reducing costs and effort compared to marker systems expressed at seedling or a later stage.

Despite of the various advantages of *R1-nj*, selection of haploid kernels is labor intensive and does not work equally well for all donors. The shape of the kernel (flat vs. round) affects the ability to see embryo coloration, as does the level of transparency of the seed coat, which is overlaying the embryo. Currently, sorting of haploids is exclusively executed by skilled labor.

The challenge for commercial breeders is to sort through large numbers of kernels within a short harvest and planting window between seasons, which may lead to suboptimal outcomes in the sorting process: 1) this task is extremely repetitive which leads to fatigue and mistakes, 2) a large workforce is required during a brief period, and 3) variation in kernel shape and expression of *R1-nj* between donor populations may lead to varying false positive and false negative rates in haploid kernel detection.

The human eye is only able to detect wavelengths between 380 – 780 nm which limits the ability to detect subtle coloration differences. The speed at which a person can sort massive amounts of kernels is limited, prompting desire for automation of the haploid selection process. Though no fully implemented system is being commercially used, several other pilot studies have been published using other markers to discriminate between the haploid and diploid fractions using instrumentation. Traits such as the difference between the embryo weights of the haploid and diploid seed (Smelser et al., 2015), spectral differences using NIRS and SIMCA (Jones et al., 2012), fluorescence imaging (Boote et al, 2015), and oil content (Melchinger et al., 2015) are all being tested for their utility as automated selection criteria. Each method has its strengths and drawbacks. The weight, NIRS, and fluorescence methods all utilize existing markers, while the oil content method requires the development of new high oil haploid inducers. The development of a high oil inducer is not a trivial matter as oil content is a quantitative inherited trait and can be affected by environmental conditions as well as context dependency of the germplasm. Herein, we describe an approach based on the VideometerLab 3 spectral imaging system, which has shown great success in other seed based assays. The ability to automate haploid – diploid kernel discrimination would allow for a substantial decrease in costs and increase in efficiency of

the maize DH system and any other DH system in which a seed color based selectable marker is used. It is important to note that this process does not necessarily need to be 100% accurate. The ability to enrich haploid kernels to >80% would still be a desirable outcome saving both money and time.

The objectives of this study were, to (i) evaluate the utility of the VideometerLab 3 system to discriminate between haploid and diploid seed, (ii) test the system on several genotypes with varying difficulty of manual sorting, and (iii) employ DNA marker assays for confirmation of haploid-diploid discrimination.

Methods

Germplasm

For this experiment, induced kernels were produced in the summer of 2012 at the Iowa State University Agronomy and Agricultural Engineering Research Farm in Boone, IA. As part of a larger experiment 120 inbred lines were planted in single row, 5.48 meter plots on 45.72 cm row spacing at a density of 60,000 plants per hectare. All plants were shoot bagged and detasseled to reduce foreign pollen contamination. When all plants in the plot reached approximately 50% silking, bulk pollen from the maternal haploid inducer F_1 'RWS/RWK-76' (Rober et al., 2005) was used to pollinate all plants in the row. At maturity, all ears in the row were bulk harvested and shelled. As part of a separate experiment, each plot was visually sorted for haploid and hybrid kernels to determine induction rate. Six inbred lines were selected to be used in this experiment to test the ability of the Videometer system to select haploid kernels using the *R1-nj* marker in the kernel.

The six inbred lines selected are as follows: 'PHR36', a DuPont Pioneer expired PVP white semi dent inbred from the non-stiff stalk heterotic group; "PHT77', a DuPont Pioneer expired PVP yellow dent inbred from the non-stiff stalk heterotic group; 'PHK35', a DuPont Pioneer expired PVP yellow dent inbred from the stiff stalk heterotic group; 'B47', a DuPont Pioneer expired PVP yellow dent inbred from the stiff stalk heterotic group, developed from the Iowa State public inbred line 'B37'. 'NK792' is an inbred line developed by Northrop, King and Company. A PVP certificate was applied for but withdrawn for 'NK792'. 'MS198' is a yellow semi dent public inbred developed by Michigan State University. Detailed information for each of these lines can be found in the PVP certificates which can be accesses through the USDA's Germplasm Research Information System (GRIN) (http://www.ars-grin.gov/npgs/). From each of the six inbred lines, 100 random haploid, and 100 random diploid kernels were selected and used for analysis.

Sample preparation and image acquisition

From each inbred, 50 kernels were randomly selected and placed in a 9 cm diameter petri dish with the embryo facing up. In some cases, it was difficult to orient the kernel with the embryo up due to the shape of the kernel. However, for most of the kernels it was possible to place them correctly with the embryo facing up.

For image acquisition, the VideometerLab 3 system was utilized. This instrument acquires multispectral images of the reflectance from the surface of maize kernels. Using strobed LED technology the VideometerLab 3 combines measurements at 19 different wavelengths into a

single high resolution spectral image. Each pixel in the image is a reflectance spectrum which includes wavelengths ranging from ultraviolet, to visual, into the near infrared spectrum (thus outside the range visible to the human eye). In addition to the illuminated wavelength, 4 filters were included in the analysis for measuring fluorescence from the kernel surface (cutoff at 400, 500, 600, and 700 nm).

In the first step, the instrument was calibrated, and the light setup prepared to match the samples in such a way that the captured images contain an as wide a dynamic range as possible with a minimum of saturated pixels. Next, petri-dishes with kernels were placed in the VideometerLab 3 instrument and images acquired for each petri-dish. During image acquisition, images were stored on a hard drive, available for further image processing. Images were taken with 2056x2056 pixels and 79 bands (regular reflectance, plus fluorescence measured with the above mentioned four cutoff wavelengths). Each image was 1.24 Gb in size.

Detection of maize kernels

In order to properly identify separate kernels, each must be segmented in the image into so-called BLOBs (Binary Labelled Objects). This process is diagrammed in Figure 1 where the image (1.1) is divided into either foreground or background (1.2). Next, the multispectral information available in each pixel in the labelled areas is used to create a linear model (CDA – canonical discriminant analysis) (Olesen et al., 2011), that ensures the canonical discrimination function will “score” high when pixels look the same as a kernel and “score” low when pixels look like the background (1.3). Finally, this score is used to do the final segmentation of the kernels (1.4) into segregated objects (1.5).

Haploid vs diploid score

Once a list of BLOBs is created, models are constructed of the diploid embryo. Due to aforementioned differences in expression of the *R1-nj* marker, colored embryos differ between inbreds. Thus models need to be generated for each of the inbreds. This is done in a similar way as for segmentation of kernels described in the previous section (Figure 2). First, regions are labelled inside the kernels (2.1 and 2.2), such that the diploid embryos have one label, and the endosperm and haploid embryos have separate labels. As before, a model is constructed (2.3) that yields a high discriminant score when the pixels "look" like a diploid embryo and low when not (2.4 and 2.5).

Extracting the haploid vs diploid score and haploid identification

Since the discriminating feature between haploid and diploid kernels is solely contained within the embryo of the kernel, the extracted measure was restricted to the embryo of the kernel. In order to do this, the "surface" (or region of interest) of the embryo is detected and isolated. Once this region is isolated, the diploid score is extracted from only this region for each kernel (Figure 3). For each set of 100 induced kernels from each of the six inbreds, the above procedures were followed and for each of the genotypes the identification rate was estimated for three different approaches: 1) for each kernel the diploid score was used to evaluate, whether it is more similar to other diploid or haploid kernels. 2) For each kernel a score is created by combining all the diploid scores (for all genotypes) using CDA. 3) In addition to the aforementioned criteria, addition of more features related to shape and texture of the kernels was used in the model.

Testing of haploid vs diploid scores on seed genotypes

To test the system, a random sample of 20 kernels from the haploid and diploid fraction was placed on two separate petri dishes (See Figure 4). Ten of the kernels in each of the petri dishes were used to train the model for the specific genotype both for fluorescence and for visual light. Once the model was trained by selection of the optimally discriminant wavelength for that genotype, it was used to generate a haploid vs diploid score for the remaining ten kernels on the plate. Individual kernels received unique scores. These individual kernels were then subjected to marker analysis for a validation of the haploid vs diploid state.

Marker analysis for confirmation of haploid vs diploid identification

As previously mentioned, the *R1-nj* color marker is not perfect. Expression of this marker is variable in both embryo and aleurone. Other issues such as kernel size and shape, time between pollination and harvest and disease pressure create more variability in the visibility of the coloration to the human eye. Thus, to definitively confirm the haploid vs diploid identity of the kernels, all kernels were planted in a greenhouse tray for DNA extraction from leaf tissue. It should be noted that a 'seed chipping' approach is not possible since the endosperm is a successful fertilization between the donor plant and the inducer in all haploid kernels. The triploid endosperm, would therefore, always be a hybrid. At the 2-3 leaf stage, tissue samples were collected for DNA extraction. Prior to this analysis, the six lines, and the inducer used for pollination were used to identify polymorphic markers that could be used to positively identify hybrids between the inducer and the line and also identify the line itself (Supplemental Table1). DNA was extracted using a CTAB protocol (Stewart et al., 1993) with plant tissue which was

flash frozen with liquid nitrogen. DNA was then separated using isomyl-alcohol:Chloroform solution and dissolved into ethanol. DNA was diluted to 20ng/μL for PCR.

Results

Identification of haploids vs hybrids using fluorescence

For each individual genotype, a specific wavelength produced an optimally discriminating value for that specific genotype. In Figure 7, boxplots for each of the six genotypes were generated to show the distribution of kernel scores for the haploid and diploid fractions of each genotype. In this figure, the optimal score was used based on the model generated for each genotype, thus producing the most discriminating values possible. As seen in both the boxplots of Figure 5, and Table 1, significant differences were obtained in all genotypes except for NK792 at the 0.05 significance level. All tests were conducted using a Welch two-sample T-test (Welch, 1938) as implemented in the R function 't.test'. The Welch two-sample T-test is appropriate as the sample sizes are small and the variances of the two fractions are not equal. Using Satterthwaite's approximation, percentage points of the *t* distribution were modified using an estimation of degrees of freedom based on the separate individual sample variances instead of a pooled variance estimates. More variation within genotypes was found for genotypes PHT77 and NK792. These genotypes also have the least significant differences between the haploid and diploid fractions. This is not surprising, as these genotypes were the most difficult to sort by hand.

The effect of using the correct model for a specific genotype can be seen in Figure 6, and Table 2. For this set of boxplots, PHT77 was sorted using wavelengths optimized for all six genotypes. For example, PHT77.1 uses the wavelength that is optimized for PHR35. In the boxplots, it can

be seen that the variance within genotypes increases when the non-optimal wavelength is used. In this case, the wavelength that is optimized for PHT77 (PHT77.2) and PHT77.3 and PHT77.4 produce significant differences. A similar effect can be seen for all genotypes when the non-optimal wavelength is used (data not shown).

Identification of haploids vs hybrids using visual light

In addition to using non-visible light wavelengths, the kernels were analyzed using wavelengths in the visible light spectrum. Again, each genotype had an optimal wavelength which produced the most discriminating score for differentiation of haploids vs hybrids. In Figure 7 and Table 3, the results of this analysis are shown in boxplots and significance values for t tests of each genotype. Differences between haploids and hybrids were significant for all genotypes except for NK792. With visual light, similar results were obtained, when non-optimal wavelengths were used (Figure 8, Table 4) with significant differences for PHT77 only when using this genotype's specific wavelength (PHT77.2) or the wavelength for PHR35 (PHT77.1). Similar results were obtained with the other genotypes (data not shown).

In a maize breeding program, a breeder might evaluate 10,000-40,000 DH lines per season. If 200 lines are evaluated per cross, then each breeder could be requesting DH lines from 50-200 unique F_1 crosses that are induced. There would be great utility in having a 'global wavelength' which can be used across all germplasm in the breeding pool. As mentioned, this study produced a unique optimized wavelength for each genotype. To evaluate the possibility of a 'global wavelength', all comparisons were made between haploids and diploids within each genotype for every wavelength (Table 5). In PHR35, PHK35, PHB47, and MS198 there are significant

differences between the haploid and diploid fractions for every wavelength. However, PHT77 and NK792 do not show significant differences for all wavelengths. Based on this information, there would be the possibility to use a global wavelength, however, each genotype would need to be visually checked to ensure that it is being properly sorted.

Marker analysis verification of haploid vs diploid scores

For this analysis, it was assumed that all non-germinating seed were of the haploid fraction. Haploid seed produce lower germination rates on average, as these embryos are weaker since they lack half of their genetic information (Prigge et al., 2011). Accuracies between the VideometerLab 3 score and molecular marker identification for the six genotypes ranged from 40% to 100% (Table 6), with the lowest accuracies for those genotypes, which were most difficult to sort by hand (see Supplemental Table 1 for a full list of scores). Manual sorting for genotypes PHR35, PHB47, and MS198 was 100% accurate based on the haploid vs hybrid score compared to marker analysis. Haploids were detected for PHT77 with 40%, for PHK35 with 70%, and for NK792 with 50% accuracy.

Discussion

Success of identification

The overall objective of this study was, to determine whether the Videometer system can be used to accurately sort haploid and hybrid seed in maize. Sorting of haploids from a maize DH program is a very time consuming and tedious process that can take thousands of hours of labor to complete for a single average sized breeding program. Many of the sorting efforts are out sourced to countries where winter nurseries are located as labor is usually cheaper and the seed

can then be readily available for planting. This study shows that there is a significant potential to utilize the Videometer system for the automation of sorting of haploid seed for maize DH programs utilizing the *R1-nj* marker system in their *in vivo* induction program. As is seen in the results, some genotypes proved difficult to sort. The six genotypes were classified into two different groups 1) easy to visually sort, and 2) difficult to visually sort. The genotypes that were easy to visually sort are PHR35, PHK35, PHB47, and MS198. The genotypes difficult to sort were PHT77, and NK792. This is a common problem seen in all maize DH programs as the expression of *R1-nj* is dependent on the genetic background of the donor population as well as environmental factors. In the case of this study the background within genotypes is uniform as all genotypes were produced from inbred lines. A suitable next step, will be to consider segregating donor populations to see what effect this has on the sort. Based on the results, it is clear that the use of the optimal wavelength for a specific genotype is important. When considering a typical maize breeding program, it is likely that the majority (at least in the U.S.) of the germplasm will be yellow dent corn. Most yellow dent corn which is properly pollinated will provide good expression of R1-nj. However, it remains possible that certain combinations could provide modification of the expression of R1-nj since this is not a trait which is selected for so any modifier loci should segregate in the germplasm. Modifications to the expression could make visual and automated sorting more difficult. It is therefore most likely that breeding programs would need to classify their induced seed into two groups as was done in this paper: those which are easy to visually sort, and those which are difficult to visually sort. As shown in this study, those which are easy to visually sort can be accurately sorted using the VideometerLab 3 system. Those which are difficult to sort could be run through the system multiple times to, at least, enrich the fraction of haploid seed in the mix which would still provide an advantage. It is also

crucial to train the model with accurate visual sorts. If the visual sorts used to train the model are poor, then the model will poorly discriminate the fractions for that genotype as was the case for NK792. Visual scores (Supplemental Table 1) for NK 792 identified the haploid and hybrid fractions as they were sorted. However, marker analysis showed that only four of the haploids were correctly identified. In previous sections, it was noted that NK792 produced the least significant values between the two fractions. Based on visual and marker scores, the haploid fraction contained five hybrids. It is thus likely, that the training kernels also contained a similar fraction of hybrids. Thus when the model was trained, half of the kernels were incorrectly classified producing a poor model to sort from. This explains the poor discrimination found for NK792. This will continue to pose a challenge, but it was promising to see that the automated scores corrected for a few visual sorting mistakes. The fitting of a global model would eliminate the need for manual sorting for each different genotype prior to sorting. However, it is shown in Table 5 that only the genotypes in the easy to sort group (PHR35, PHK35, PHB47, and MS198) are sortable using the optimized wavelengths from other genotypes. One aspect to consider, however, is that global models may work better within the germplasm used in specific breeding programs. For example, a breeder may be able to generate a global model for their stiff stalk dent germplasm, and a separate model for their non-stiff stalk germplasm. This requires further testing, but with growing interest in the possibility of automation should be possible in the near future.

Challenges and automation

A major challenge of working with maize kernels for imaging is that the kernels are of an irregular shape and do not always lay flat depending of many factors including the number of

kernels on the ear, and the location of the specific kernel on the ear with flatter kernels occurring with complete pollinations in the middle of the ear and rounded kernels occurring in incomplete pollinations and on the ear base and tip. In order to use the system as it currently works, the kernels must be positioned with the embryo facing upward which proved challenging. A system will have to be devised where the kernel can be displayed to the optics in a consistent and accurate position for imaging. Discrimination of the embryo from the rest of the kernel is a useful aid to discrimination of the two fractions as this is the only part of the kernel which has a detectable difference between the two fractions.

Currently, the positioning of the kernels onto a petri dish is not a high-throughput method which would allow for the sorting of the millions of kernels which would be needed. Now that the system has been shown to be discriminatory between hybrids and haploids, it will be important to design and automated system which can feed a large quantity of seed to the imaging system (e.g., through a conveyor or channel system) which would then subsequently sort the seeds into two fractions using robotic picking or some kind of pneumatic or mechanical gate system. It is important to note, that a 100% accuracy rate is not necessary. A rate of 10% hybrids in the haploid fraction would be acceptable, as these can be cut out of the field easily. It is most important to identify all haploids in the seed genotype, even if this means that some hybrids are misclassified due to the setting of the sort threshold. Losing haploids into the hybrid fraction would be undesirable as haploids only occur at, on average, a 10% rate in an induction cross and each seed can be critical to the production of enough DH lines for breeders to evaluate per cross.

Comparison to other methods

As mentioned in the introduction, other pilot studies have been conducted which evaluate the ability to use an automated system to discriminate between haploid and hybrid seed in an induction cross. The method proposed herein, as mentioned, uses the Vidometer Lab 3 system which has been documented as a useful tool for the nondestructive and automated analysis of seed phenotypes (Liu et al., 2014; Shetty et al., 2012; Olesen et al., 2011). This method is able to capitalize on the already existing marker system (*R1-nj*) and would also not require the development of a new set of optical sensors/software to handle the data. This in comparison to, for example, the previously mentioned Jones et al. (2012) which required the development of a new system for the analysis of the seeds as well as software to be adapted for its use. Though the method in Jones et al. (2012) provided a discriminative sort, there was no validation of the true identity of the haploid/hybrid kernels. The method described by Boote et al. (In press), provides the most similar method, using NIRS and fluorescence imaging. However, this method only considers on kernel at a time and while discussion of automation was provided, no current method exists for that system, while for the VideometerLab 3 system a method of automated seed feeding exists (shown here: ftp://videometerlab:multispec@www.videometer.com /Videos/2014_July_VideometerLab_AutoFeeder.AVI). The method described in Smelser et al. (2015) uses the weight of the haploid and hybrid embryos as a marker to discriminate between the two seed fractions. In this study visually selected kernels were weighed and total kernel weights were compared as a discriminative marker. Only two of the six genotypes produced significant differences. Automation of such a system could prove difficult since it would rely on single kernel weights. Also, it is unclear what the effect of kernels of different size would have. Kernels on the tip and base of the ear are sized differently than those in the center which will affect the overall weight of the kernel. There may also be loci which control the size of the

embryo which segregate in the germplasm affecting the accuracy of the sort. Again, no validation of the identity of the visual sort was provided. Finally, the method proposed by Melchinger et al. (2015; 2014; 2013) uses oil content as a marker for discrimination between haploid and hybrid seed. While detection of oil content has the potential to be automated on a single kernel basis, and the ability to discriminate the two fractions was shown, there is a dependency on the genetic background of the material as is the case in all studies discussed. Modifier loci for oil content may segregate within the germplasm causing confounding effects of the oil content expression. The most difficult aspect of the high oil marker, is the development of new high oil inducer lines. While the VideometerLab 3 system relies on existing marker technology, using oil content would require the development of new inducers with both high oil content and high induction rate. Both of these traits are quantitative and it would not be a trivial task to increase the mean induction rate and the mean oil content simultaneously, though it can be done as they describe. This would however, make the system more expensive as it would not only require the instrumentation, but also either the development or purchase of a new high oil inducer.

Considering these methods, it seems that the VideometerLab 3 system would be the easiest to implement due to the fact that 1) the instrumentation is already designed and well tested showing success in other seed phenotypes, 2) software is already developed for this system, 3) this study shows that discrimination of haploids and hybrids is possible, and 4) it utilized the existing inducers and marker system.

Acknowledgement

This project is supported by the SEVENTH FRAMEWORK PROGRAMME of EU, Industry – Academia Partnerships and Pathways (IAPP) - Marie Curie Actions (grant no.: 324433). This project was also supported by the Iowa State University Raymond F. Baker Center for Plant Breeding.

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Tables

Table 1. Comparison between haploid and diploid fractions using fluorescence for each of the six genotypes. Comparisons were made using the Welch two sample T-test. The optimal wavelength for each genotype was used in this analysis.

Genotype	Fraction	Mean	Std. Dev.	P-Value
PHR35	Hybrid	-0.19	0.36	< 0.001*
	Haploid	-1.06	0.1	
PHT77	Hybrid	-0.48	0.35	0.0012*
	Haploid	-1.15	0.41	
PHK35	Hybrid	-0.33	0.18	< 0.001*
	Haploid	-1.15	0.17	
PHB47	Hybrid	0.03	0.29	< 0.001*
	Haploid	-0.91	0.12	
NK792	Hybrid	-0.65	0.38	0.051^{NS}
	Haploid	-0.94	0.19	
MS198	Hybrid	-0.26	0.18	< 0.001*
	Haploid	-0.92	0.26	

Table 2. Comparison between haploid and diploid fractions using fluorescence for PHT77. Comparisons were made using the Welch two sample T-test. In this table, optimal wavelengths for each of the six genotypes were used on PHT77.

Genotype	Fraction	Mean	Std. Dev.	P-Value
PHT77.1	Hybrid	-0.73	0.18	0.09^{NS}
	Haploid	-0.86	0.16	
PHT77.2	Hybrid	-0.48	0.35	0.0012*
	Haploid	-1.15	0.41	
PHT77.3	Hybrid	-0.19	0.28	0.03*
	Haploid	-0.49	0.3	
PHT77.4	Hybrid	-0.31	0.33	0.03*
	Haploid	-0.61	0.22	
PHT77.5	Hybrid	-1.69	0.2	0.33^{NS}
	Haploid	-1.77	0.19	
PHT77.6	Hybrid	-0.73	0.18	0.17^{NS}

	Haploid	-0.84	0.17	

Table 3. Comparison between haploid and diploid fractions using visible light for each of the six genotypes. Comparisons were made using the Welch two sample T-test. The optimal wavelength for each genotype was used in this analysis.

Genotype	Fraction	Mean	Std. Dev.	P-Value
PHR35	Hybrid	-0.1	0.93	<0.001*
	Haploid	-1.09	0.13	
PHT77	Hybrid	-0.72	0.24	0.002*
	Haploid	-1.09	0.2	
PHK35	Hybrid	-0.51	0.19	<0.001*
	Haploid	-1.05	0.1	
PHB47	Hybrid	-0.2	0.26	<0.001*
	Haploid	-0.92	0.13	
NK792	Hybrid	-0.81	0.24	0.051^{NS}
	Haploid	-1.01	0.14	
MS198	Hybrid	-0.45	0.18	<0.001*
	Haploid	-0.99	0.09	

Table 4. Comparison between haploid and diploid fractions using visible light for PHT77. Comparisons were made using the Welch two sample T-test. In this table, optimal wavelengths for each of the six genotypes were used on PHT77.

Genotype	Fraction	Mean	Std. Dev.	P-Value
PHT77.1	Hybrid	0.003	0.19	0.03*
	Haploid	-0.17	0.14	
PHT77.2	Hybrid	-0.72	0.24	0.002*
	Haploid	-1.09	0.2	
PHT77.3	Hybrid	-0.53	0.17	0.08^{NS}
	Haploid	-0.69	0.21	
PHT77.4	Hybrid	-0.13	0.2	0.12^{NS}
	Haploid	-0.26	0.15	
PHT77.5	Hybrid	-1.02	0.2	0.32^{NS}

	Haploid	-1.11	0.17	
PHT77.6	Hybrid	-0.72	0.12	0.17^{NS}
	Haploid	-0.79	0.1	

Table 5. Welch two sample t-test for all possible combinations using fluorescence. Each genotype was tested at all six wavelengths to observe if a global model could be applied instead of producing an optimal wavelength for each genotype.

	Wavelength					
Genotype	1	2	3	4	5	6
PHR35	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
PHT77	0.09	0.001	0.03	0.03	0.33	0.17
PHK35	<0.001	<0.001	<0.001	0.16	<0.001	<0.001
PHB47	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NK792	0.68	0.97	0.81	0.51	0.051	0.28
MS198	<0.001	0.001	<0.001	<0.001	0.002	<0.001

Table 6. Using molecular marker information that allows for the classification of a hybrid between the inbred line and the inducer and a haploid progeny of the inbred line, the accuracy of the VideometerLab 3 sort was checked.

Genotype	**Fraction**	**# correct**	**# incorrect**	**Accuracy**
PHR35	Hybrid	10	0	100%
	Haploid	10	0	100%
PHT77	Hybrid	8	2	80%
	Haploid	4	6	40%
PHK35	Hybrid	9	1	90%
	Haploid	7	3	70%
PHB47	Hybrid	10	0	100%
	Haploid	10	0	100%
NK792	Hybrid	10	0	100%
	Haploid	5	5	50%
MS198	Hybrid	10	0	100%
	Haploid	10	0	100%

Figure Legends

Figure 1. The segmentation process. Regions/pixels in the image (1) are labelled in "foreground" and "background" categories (2). The labelling is used to create a score (3) that can be used to segment the image into individual regions, each containing one kernel (4). Each region is extracted from the image (5) into so-called BLOBS (Binary Labelled Objects).

Figure 2. Identification of the diploid embryo. The diploid and haploid embryo in each kernel (1) is labelled with two different labels (2). In addition to the haploid embryo region, the surroundings, different from the diploid embryo, is included in order to create a model (3) that will score high on only diploid embryos. (4) show the resulting "diploid embryo-score" generated from the model. (5 and left side) illustrate the resulting score from a haploid embryo.

Figure 3. Before the diploid-embryo score is extracted from each kernel, we ensure that the score is extracted from the endosperm region. Hence, as for the foreground/background segmentation in Figure 6, we label the endosperm region and the rest in two labels, and create a model that we use for doing the segmentation of the embryo region.

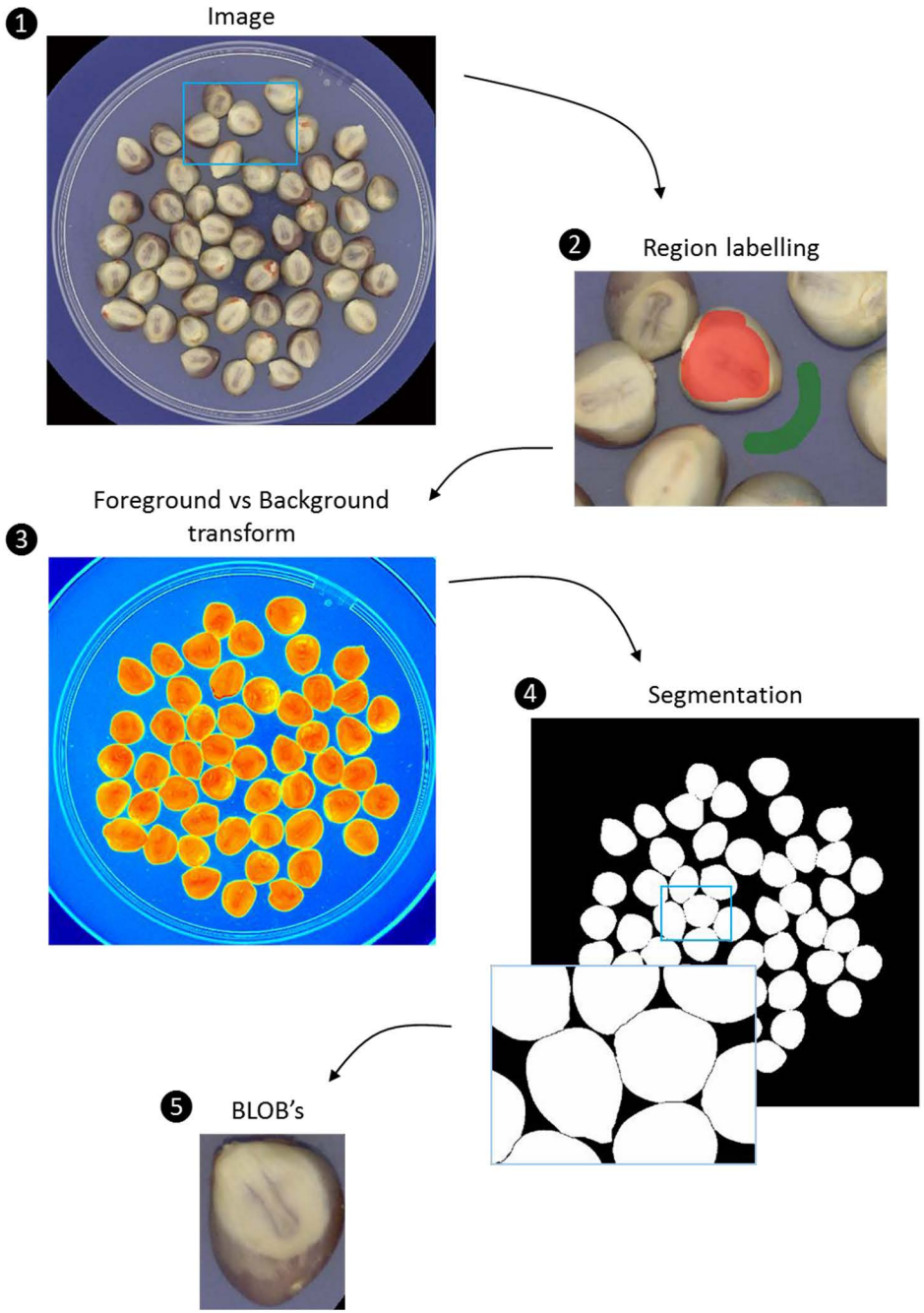
Figure 4. Experimental layout of kernels. 20 individual randomly selected kernels from the haploid and diploid fractions of each genotype were placed on a petri dish, embryo side up, for imaging. The bottom ten kernels were used to train the model, and the model was then tested on the remaining 10 kernels for each fraction.

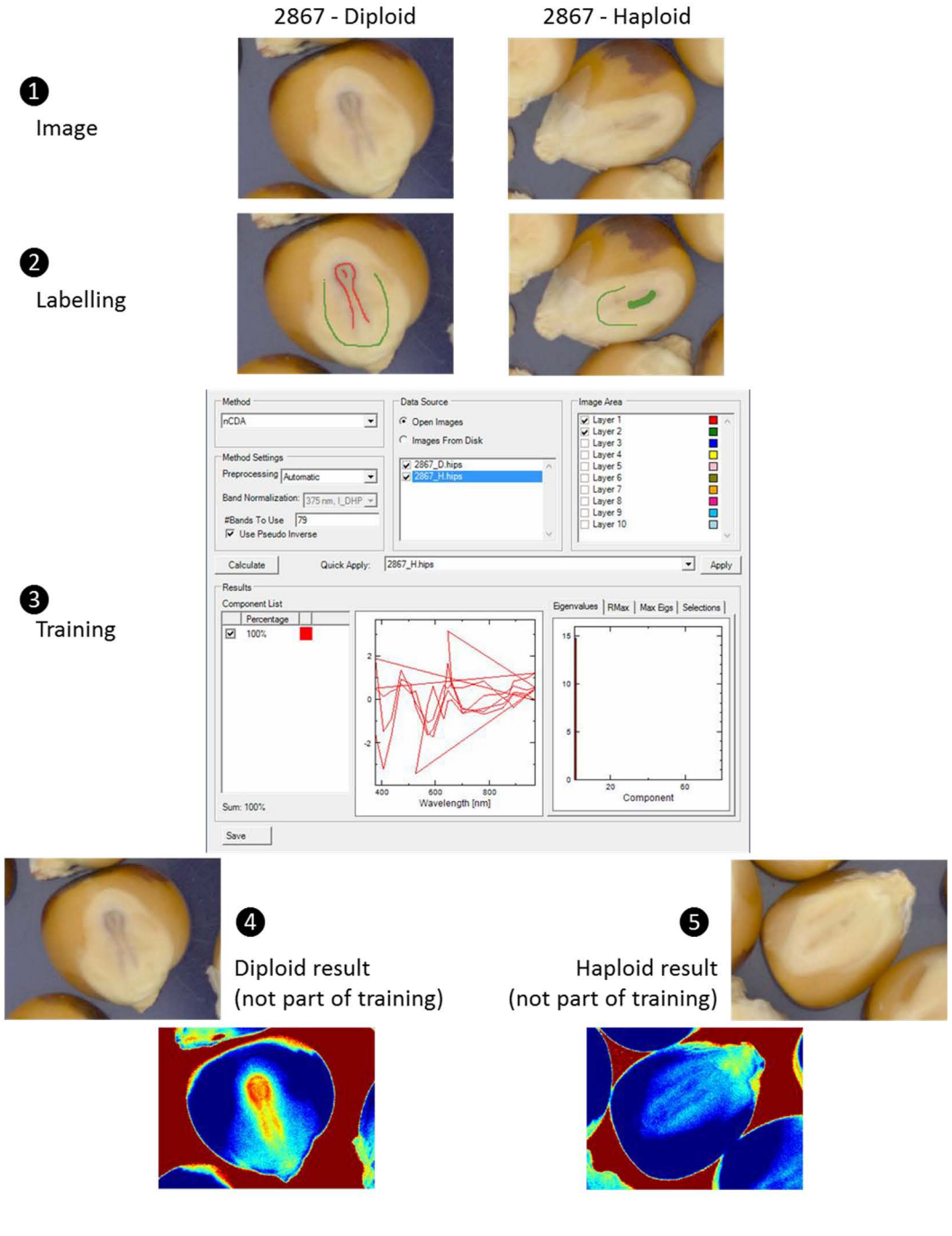
Figure 5. Boxplots showing the distribution of values for each of the six genotypes using fluorescence. As seen, some of the genotypes have small variance within each group and good separation between fractions while some have higher variance and less separation.

Figure 6. Boxplots showing the distribution of values for PHT77 using optimal fluorescence wavelengths for all six genotypes. As seen, the differentiation between haploid and diploid fractions is not as pronounced as seen in Figure 7.

Figure 7. Boxplots showing the distribution of values for each of the six genotypes using visible light. As seen, some of the genotypes have small variance within each group and good separation between fractions while some have higher variance and less separation.

Figure 8. Boxplots showing the distribution of values for PHT77 using optimal visible light wavelengths for all six genotypes. As seen, the differentiation between haploid and diploid fractions is not as pronounced as seen in Figure 9.


1
Image
2
Region labelling
Foreground vs Background
transform
3
4
Segmentation
5
BLOB's


2867 - Diploid
2867 - Haploid
1
Image
2
Labelling
3
Training
Method
nCDA
Method Settings
Preprocessing
Automatic
Band Normalization:
375 nm, I_DHP
#Bands To Use
79
Use Pseudo Inverse
Data Source
Open Images
Images From Disk
2867_D.hips
2867_H.hips
Image Area
Layer 1
Layer 2
Layer 3
Layer 4
Layer 5
Layer 6
Layer 7
Layer 8
Layer 9
Layer 10
Calculate
Quick Apply:
2867_H.hips
Apply
Results
Component List
Percentage
100%
Sum: 100%
Eigenvalues
RMax
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Selections
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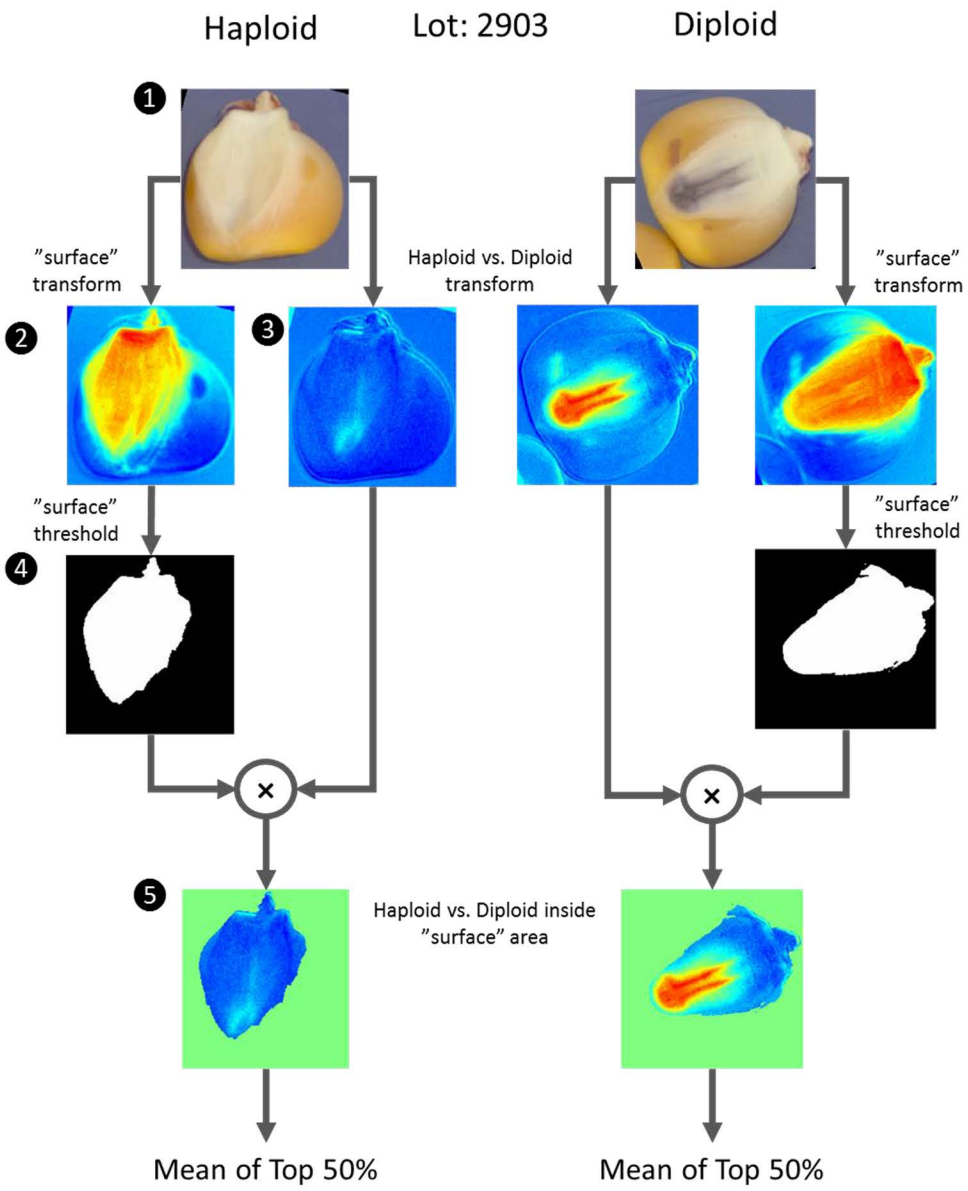
Component
Save
4
Diploid result
(not part of training)
5
Haploid result
(not part of training)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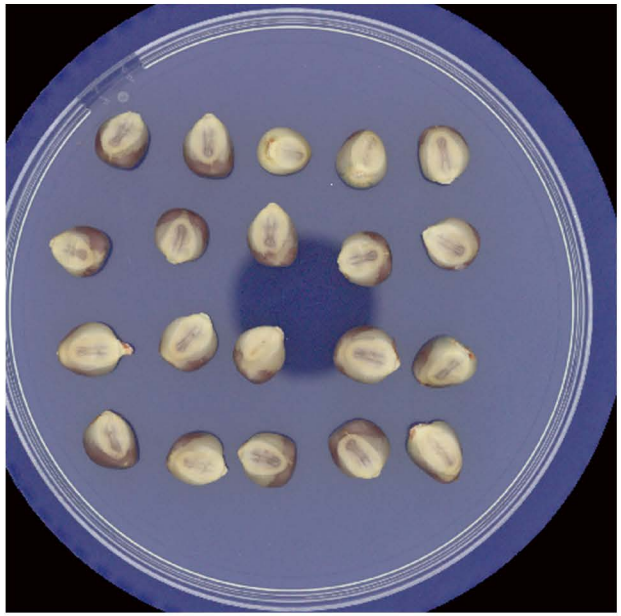
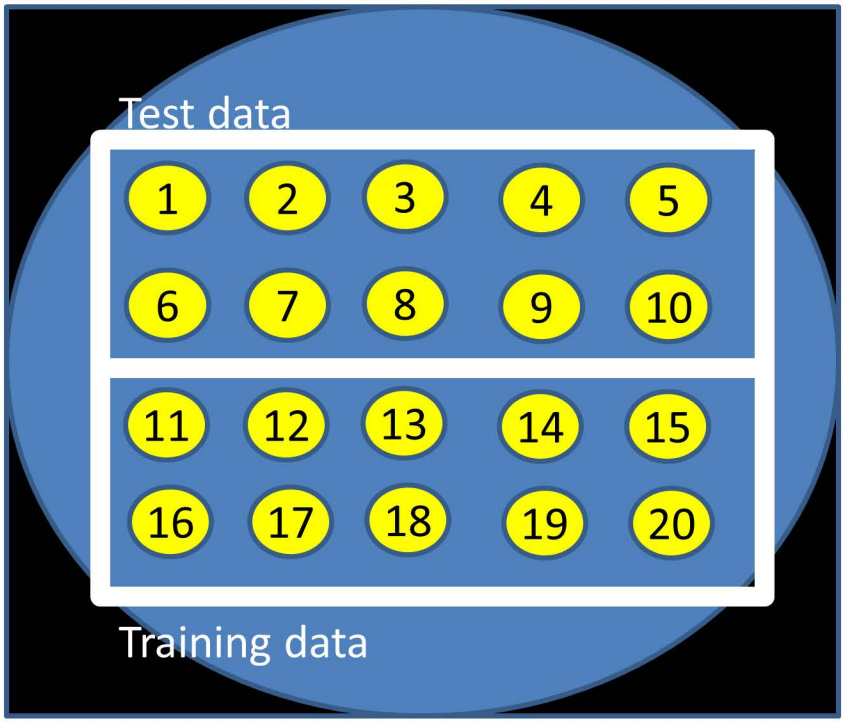
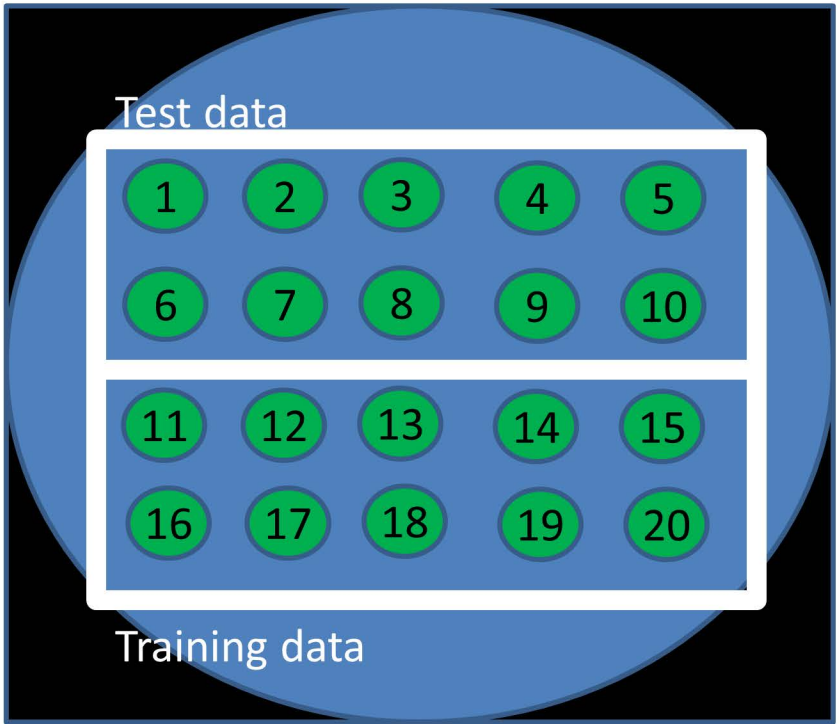
Lot: 2903
Diploid
1
"surface" transform
Haploid vs. Diploid transform
"surface" transform
2
3
"surface" threshold
"surface" threshold
4
×
×
5
Haploid vs. Diploid inside "surface" area
Mean of Top 50%
Mean of Top 50%

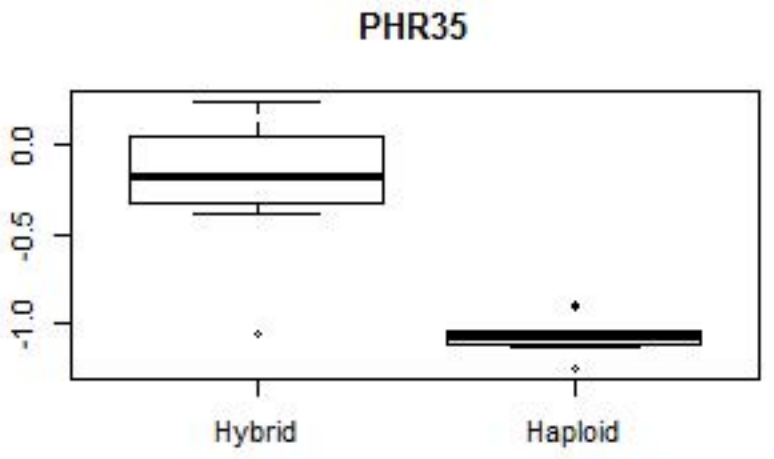


Hybrid

Haploid



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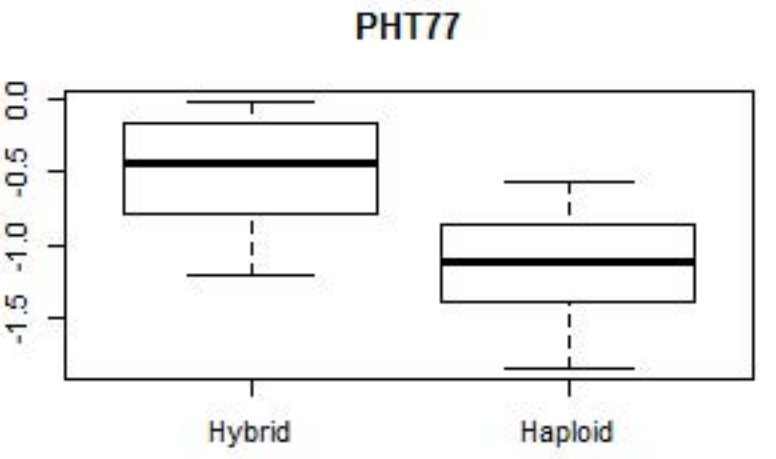
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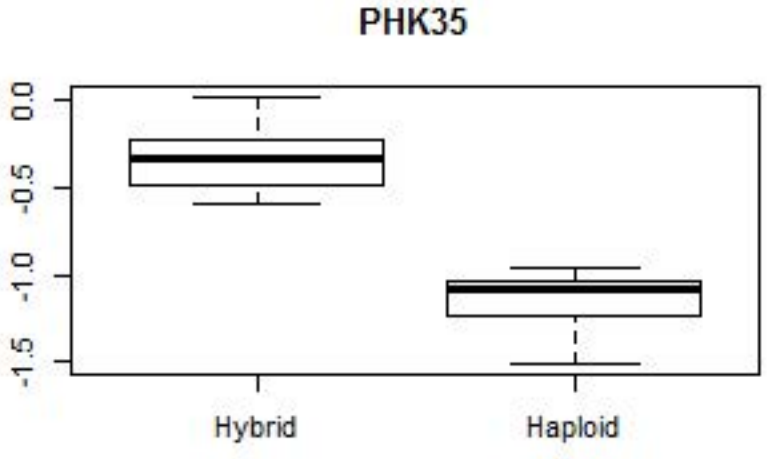
Haploid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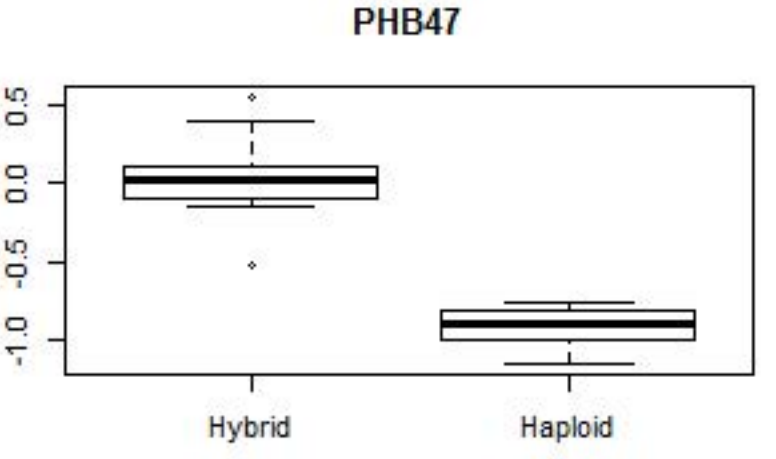
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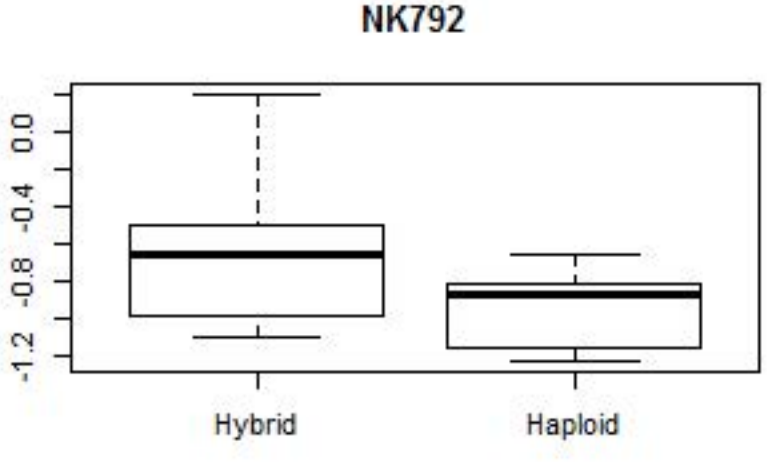
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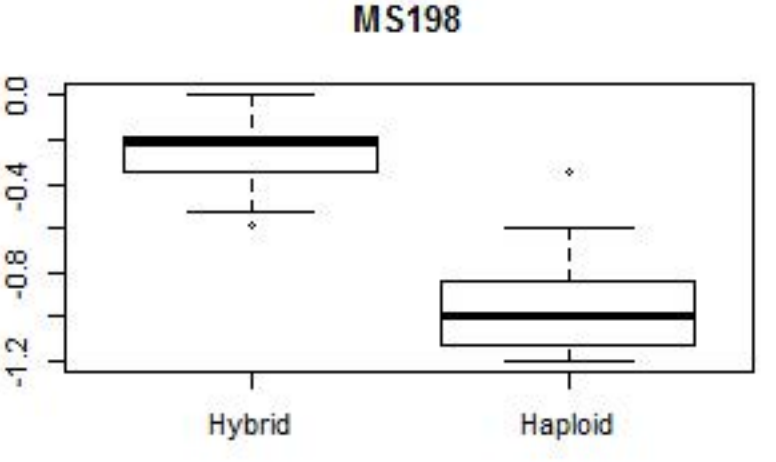
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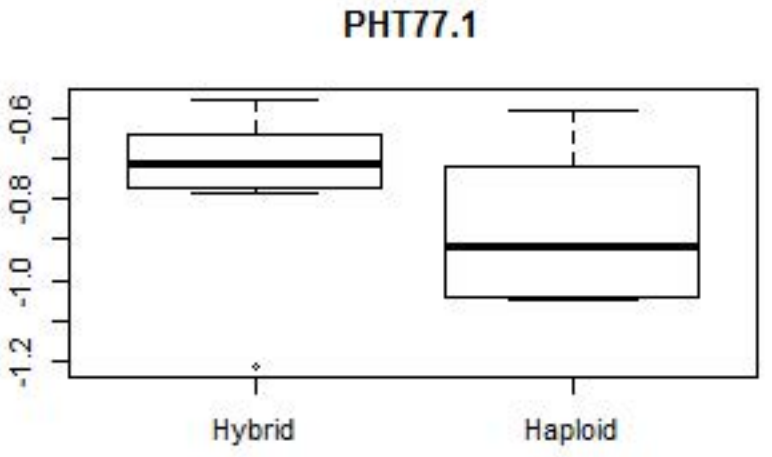
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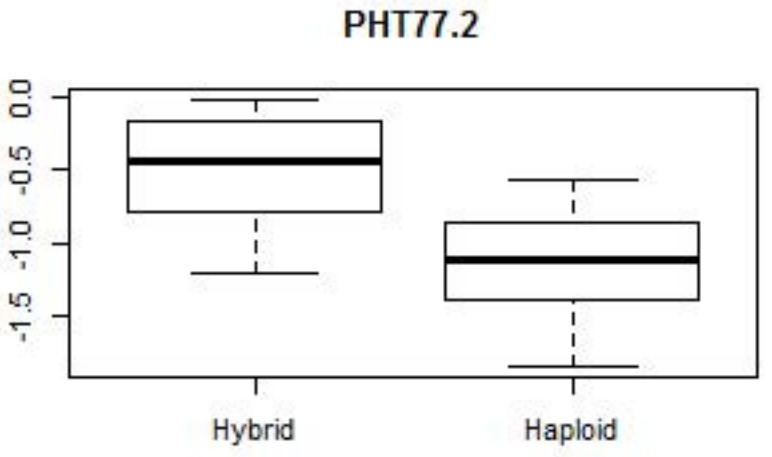
Haploid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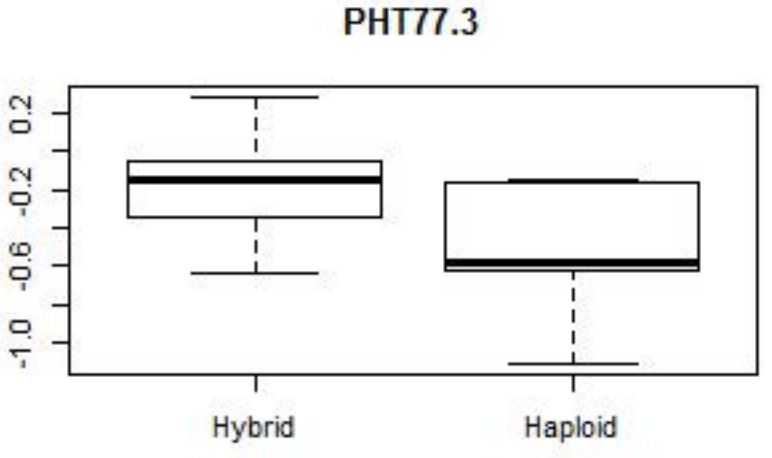
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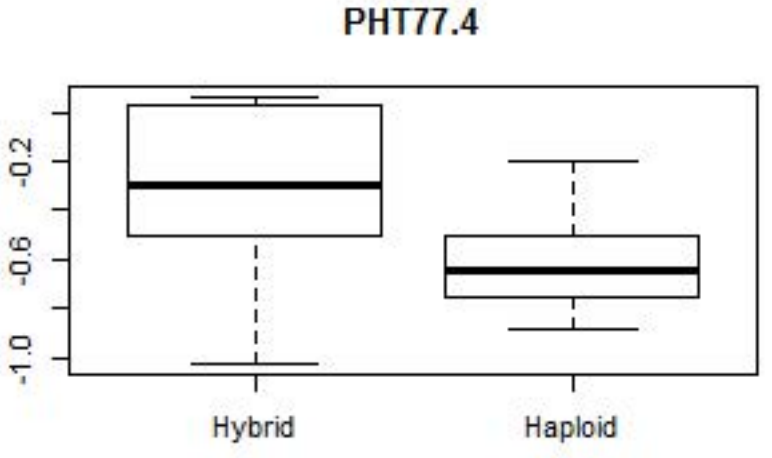
Haploid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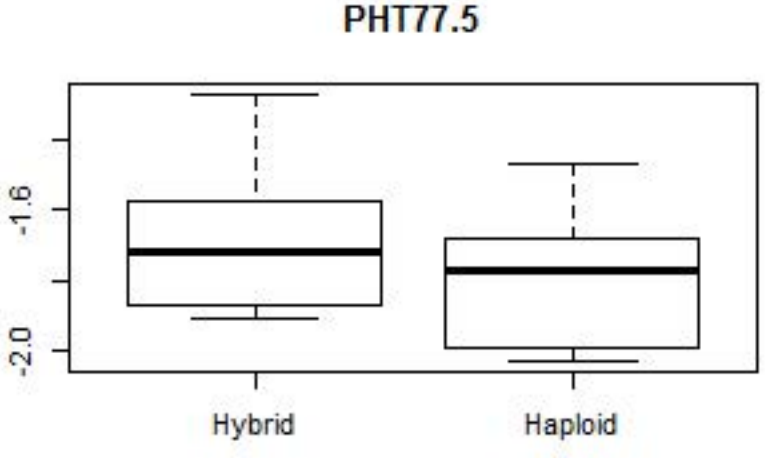
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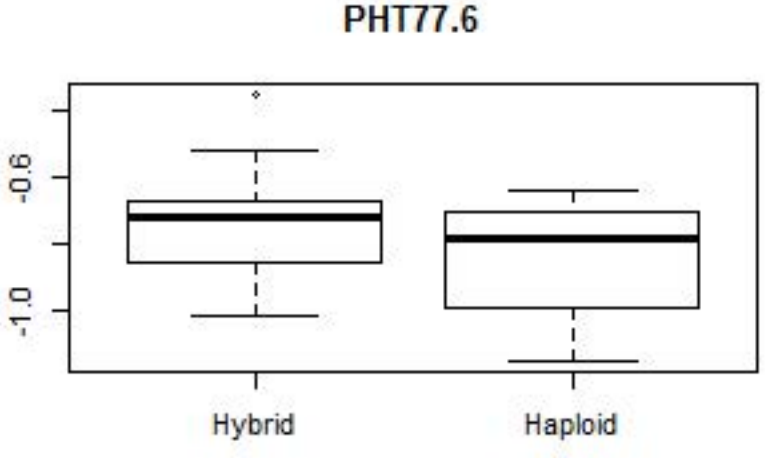
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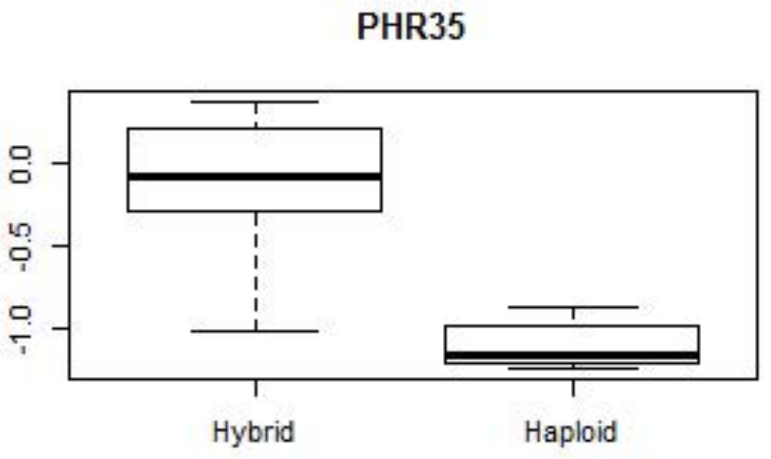
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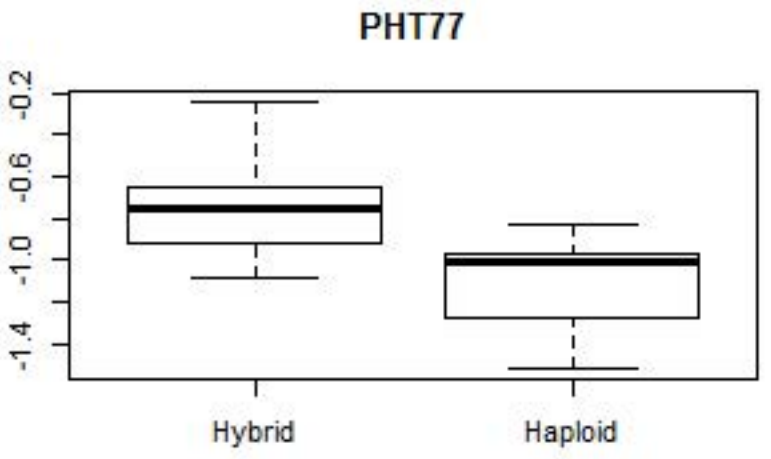
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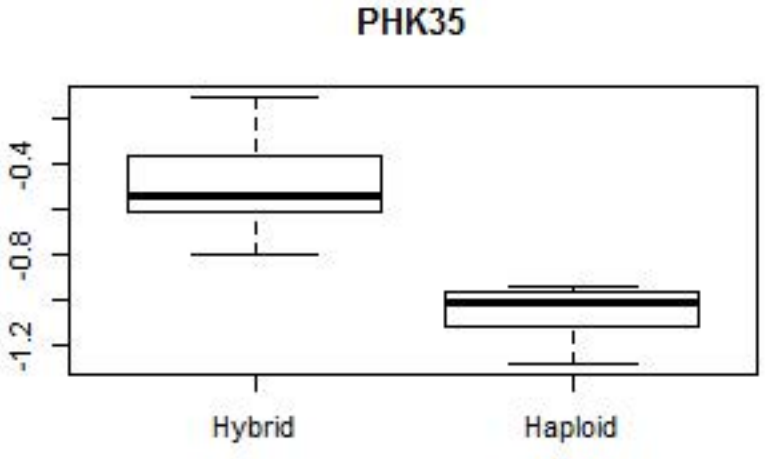
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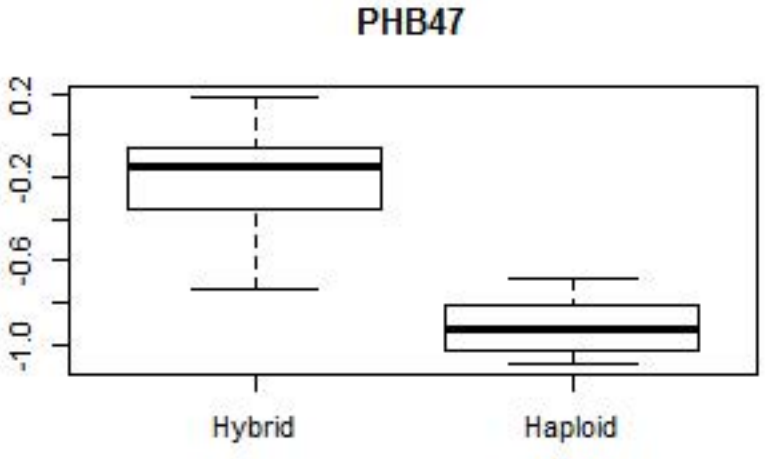
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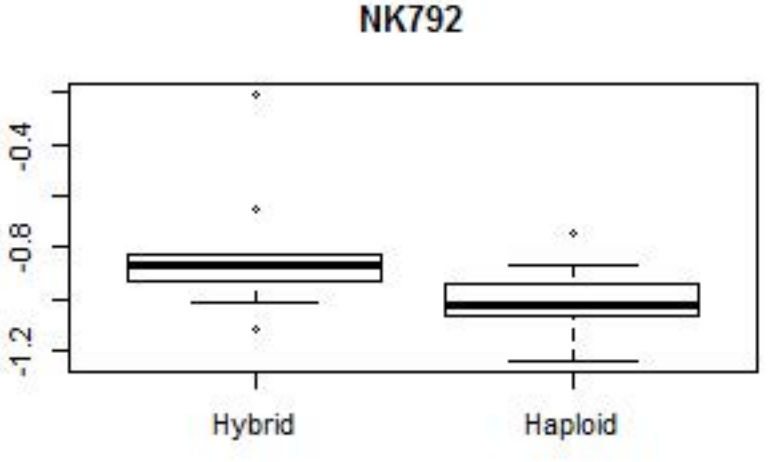
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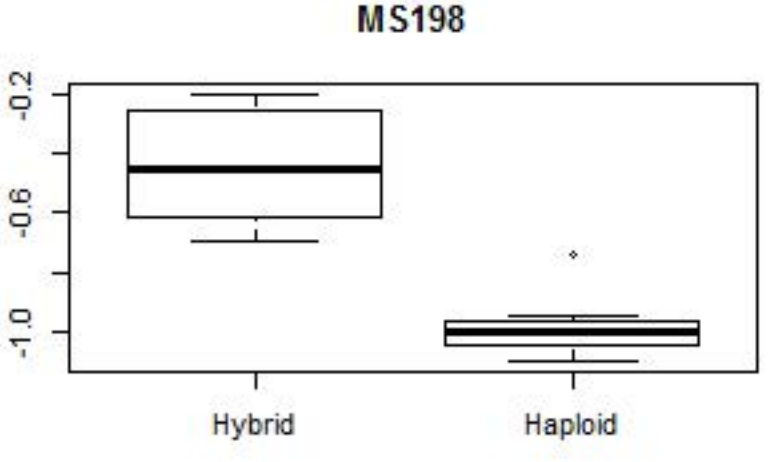
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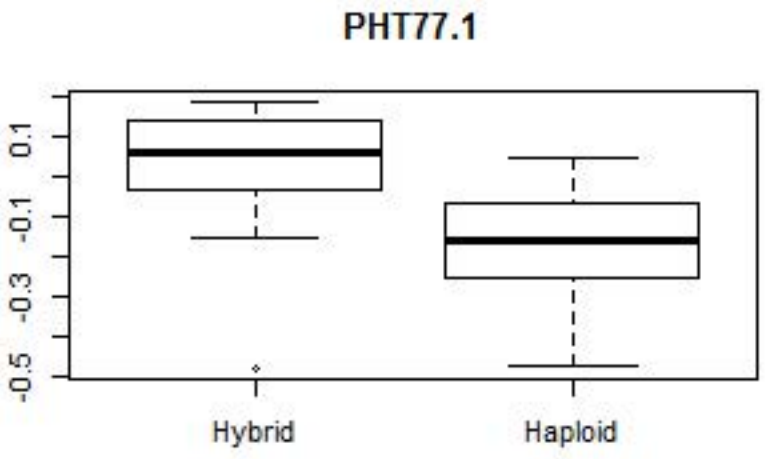
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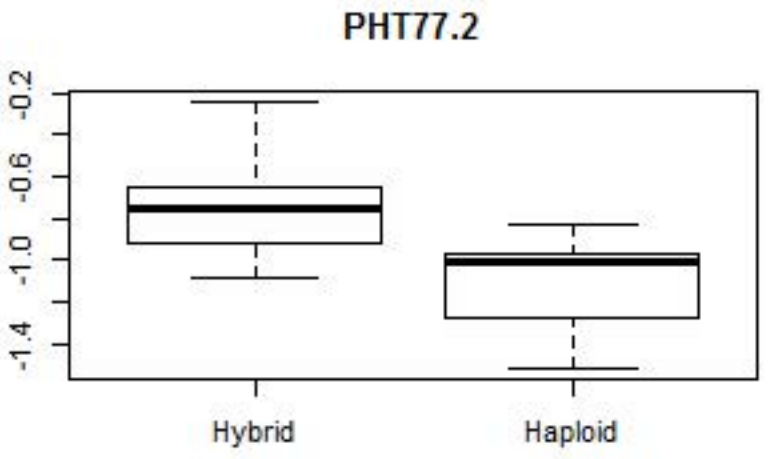
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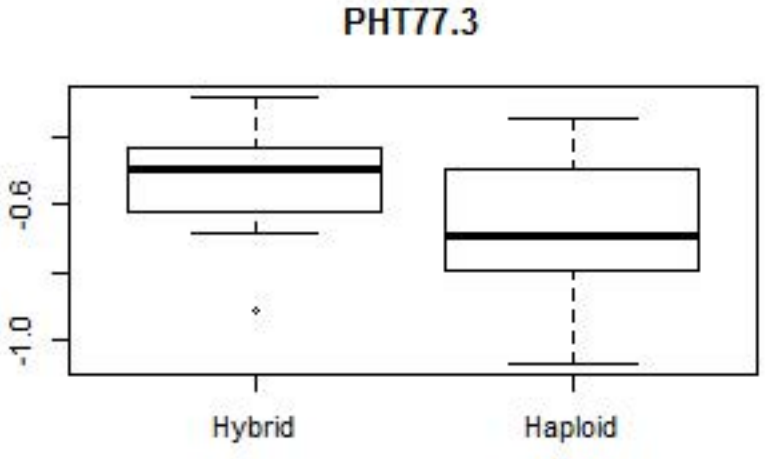
Haploid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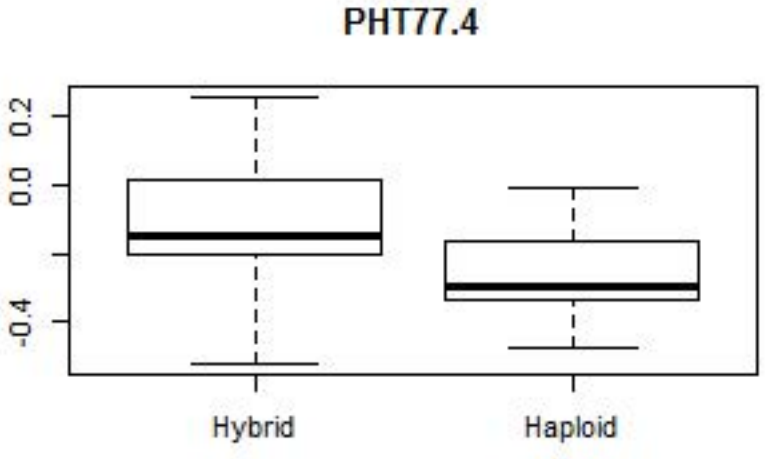
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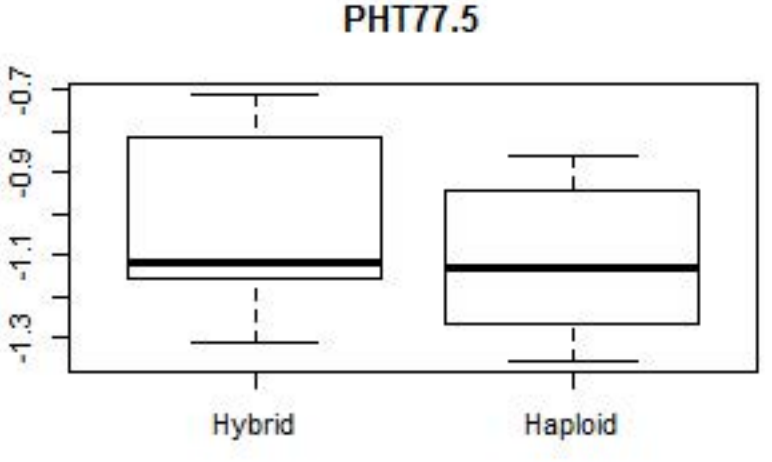
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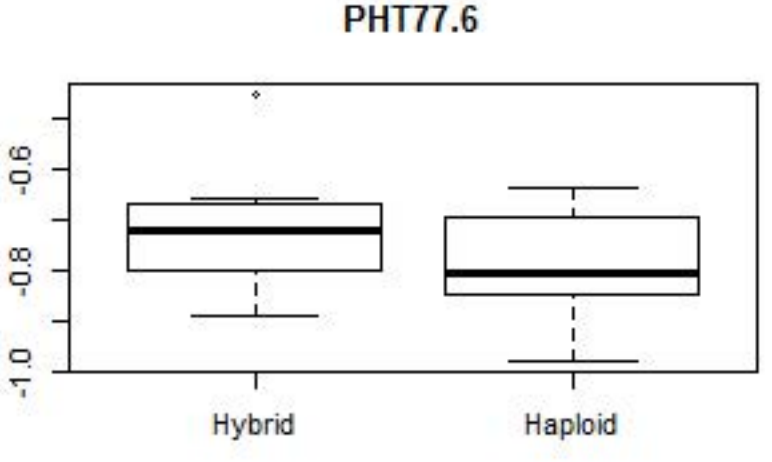
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Hybrid
Haploid

TABLE 1

Lot	Fraction	Mean	Std. Dev.	P-Value
2722	Hybrid	-0.19	0.36	< 0.001*
	Haploid	-1.06	0.1	
2820	Hybrid	-0.48	0.35	0.0012*
	Haploid	-1.15	0.41	
2867	Hybrid	-0.33	0.18	< 0.001*
	Haploid	-1.15	0.17	
2886	Hybrid	0.03	0.29	< 0.001*
	Haploid	-0.91	0.12	
2900	Hybrid	-0.65	0.38	0.051^{NS}
	Haploid	-0.94	0.19	
2903	Hybrid	-0.26	0.18	< 0.001*
	Haploid	-0.92	0.26	

TABLE 2

Lot	Fraction	Mean	Std. Dev.	P-Value
2820.1	Hybrid	-0.73	0.18	0.09^{NS}
	Haploid	-0.86	0.16	
2820.2	Hybrid	-0.48	0.35	0.0012*
	Haploid	-1.15	0.41	
2820.3	Hybrid	-0.19	0.28	0.03*
	Haploid	-0.49	0.3	
2820.4	Hybrid	-0.31	0.33	0.03*
	Haploid	-0.61	0.22	
2820.5	Hybrid	-1.69	0.2	0.33^{NS}
	Haploid	-1.77	0.19	
2820.6	Hybrid	-0.73	0.18	0.17^{NS}
	Haploid	-0.84	0.17	

TABLE 3

Lot	Fraction	Mean	Std. Dev.	P-Value
2722	Hybrid	-0.1	0.93	<0.001*
	Haploid	-1.09	0.13	
2820	Hybrid	-0.72	0.24	0.002*
	Haploid	-1.09	0.2	
2867	Hybrid	-0.51	0.19	<0.001*
	Haploid	-1.05	0.1	
2886	Hybrid	-0.2	0.26	<0.001*
	Haploid	-0.92	0.13	
2900	Hybrid	-0.81	0.24	0.051^{NS}
	Haploid	-1.01	0.14	
2903	Hybrid	-0.45	0.18	<0.001*
	Haploid	-0.99	0.09	

TABLE 4

Lot	Fraction	Mean	Std. Dev.	P-Value
2820.1	Hybrid	0.003	0.19	0.03*
	Haploid	-0.17	0.14	
2820.2	Hybrid	-0.72	0.24	0.002*
	Haploid	-1.09	0.2	
2820.3	Hybrid	-0.53	0.17	0.08^{NS}
	Haploid	-0.69	0.21	
2820.4	Hybrid	-0.13	0.2	0.12^{NS}
	Haploid	-0.26	0.15	
2820.5	Hybrid	-1.02	0.2	0.32^{NS}
	Haploid	-1.11	0.17	
2820.6	Hybrid	-0.72	0.12	0.17^{NS}
	Haploid	-0.79	0.1	

ID	Lot	Type1	Type2	Lab	Gerald	Videomete Lot	No	ID_gerald	x2722_54	x2820_54
1	2722	1	Hybrids	D	D	D	2722	1	2722_1	0.044185	0.055918
2	2722	1	Hybrids	D	D	D	2722	2	2722_2	-0.191866	-0.538942
3	2722	1	Hybrids	D	D	D	2722	3	2722_3	0.231175	0.412676
4	2722	1	Hybrids	D	D	D	2722	4	2722_4	0.188135	0.321838
5	2722	1	Hybrids	D	D	D	2722	5	2722_5	-0.380477	-0.660052
6	2722	1	Hybrids	D	D	D	2722	6	2722_6	-0.296105	-0.636864
7	2722	1	Hybrids	D	D	D	2722	7	2722_7	-0.153276	-0.420734
8	2722	1	Hybrids	D	D	D	2722	8	2722_8	-1.055690	-1.689250
9	2722	1	Hybrids	D	D	D	2722	9	2722_9	-0.039378	-0.434579
10	2722	1	Hybrids	D	D	D	2722	10	2722_10	-0.330018	-0.730793
11	2722	2	Haploids	H	H	H	2722	1	2722_1	-0.903298	-1.847237
12	2722	2	Haploids	H	H	H	2722	2	2722_2	-1.084058	-2.261714
13	2722	2	Haploids	H	H	H	2722	3	2722_3	-1.063205	-1.984627
14	2722	2	Haploids	NA	H	H	2722	4	2722_4	-1.080100	-1.897987
15	2722	2	Haploids	H	H	H	2722	5	2722_5	-0.891868	-1.254972
16	2722	2	Haploids	H	H	H	2722	6	2722_6	-1.054480	-1.830894
17	2722	2	Haploids	H	H	H	2722	7	2722_7	-1.040920	-1.675346
18	2722	2	Haploids	H	H	H	2722	8	2722_8	-1.129221	-2.063941
19	2722	2	Haploids	H	H	H	2722	9	2722_9	-1.116021	-2.316264
20	2722	2	Haploids	H	H	H	2722	10	2722_10	-1.247120	-2.016070
21	2820	1	Hybrids	D	D	D	2820	1	2820_1	-0.556127	-0.386612
22	2820	1	Hybrids	D	D	D	2820	2	2820_2	-0.772230	-0.777033
23	2820	1	Hybrids	D	D	D	2820	3	2820_3	-0.720226	-0.435458
24	2820	1	Hybrids	NA	D	D	2820	4	2820_4	-0.722485	-0.157096
25	2820	1	Hybrids	D	D	D	2820	5	2820_5	-1.213603	-1.205436
26	2820	1	Hybrids	NA	D	D	2820	6	2820_6	-0.781946	-0.781700
27	2820	1	Hybrids	D	D	D	2820	7	2820_7	-0.641192	-0.440081
28	2820	1	Hybrids	D	D	D	2820	8	2820_8	-0.559969	-0.026707
29	2820	1	Hybrids	D	D	D	2820	9	2820_9	-0.687442	-0.558935
30	2820	1	Hybrids	D	Contanm	D	2820	10	2820_10	-0.704361	-0.123988
31	2820	2	Haploids	D	H	H	2820	1	2820_1	-0.684468	-1.048942
32	2820	2	Haploids	D	H	H	2820	2	2820_2	-0.856719	-1.742013
33	2820	2	Haploids	NA	H	H	2820	3	2820_3	-0.581664	-0.691223

34	2820	2	Haploids	D	H	H	2820	4	2820_4	-0.718833	-0.566356
35	2820	2	Haploids	NA	H	H	2820	5	2820_5	-0.958261	-1.118061
36	2820	2	Haploids	D	H	H	2820	6	2820_6	-0.936116	-1.111221
37	2820	2	Haploids	D	H	H	2820	7	2820_7	-1.041133	-1.839460
38	2820	2	Haploids	D	H	H	2820	8	2820_8	-0.892808	-1.138649
39	2820	2	Haploids	H	H	H	2820	9	2820_9	-1.048313	-1.394191
40	2820	2	Haploids	H	H	H	2820	10	2820_10	-1.042698	-0.862853
41	2867	1	Hybrids	NA	D	D	2867	1	2867_1	-0.399056	-1.134822
42	2867	1	Hybrids	D	D	D	2867	2	2867_2	-0.690880	-1.285520
43	2867	1	Hybrids	D	D	D	2867	3	2867_3	-0.704070	-1.424087
44	2867	1	Hybrids	D	D	D	2867	4	2867_4	-0.498913	-1.037448
45	2867	1	Hybrids	D	D	D	2867	5	2867_5	-0.754737	-2.249824
46	2867	1	Hybrids	D	D	D	2867	6	2867_6	-0.730126	-1.576810
47	2867	1	Hybrids	D	D	D	2867	7	2867_7	-0.368353	-1.118113
48	2867	1	Hybrids	D	D	D	2867	8	2867_8	-0.747698	-2.060507
49	2867	1	Hybrids	D	D	D	2867	9	2867_9	-0.830002	-1.665012
50	2867	1	Hybrids	D	D	D	2867	10	2867_10	-0.736490	-1.280903
51	2867	2	Haploids	H	H	H	2867	1	2867_1	-0.973732	-2.325521
52	2867	2	Haploids	D	H	H	2867	2	2867_2	-0.865958	-2.217205
53	2867	2	Haploids	H	H	H	2867	3	2867_3	-1.076152	-2.563910
54	2867	2	Haploids	NA	H	H	2867	4	2867_4	-0.926848	-2.564159
55	2867	2	Haploids	H	H	H	2867	5	2867_5	-1.001287	-2.240811
56	2867	2	Haploids	D	H	H	2867	6	2867_6	-1.054224	-2.566566
57	2867	2	Haploids	H	H	H	2867	7	2867_7	-0.884846	-2.249028
58	2867	2	Haploids	H	H	H	2867	8	2867_8	-1.158947	-3.063361
59	2867	2	Haploids	D	H	H	2867	9	2867_9	-1.080047	-2.491242
60	2867	2	Haploids	H	H	H	2867	10	2867_10	-1.068741	-2.192089
61	2886	1	Hybrids	D	D	D	2886	1	2886_1	-0.526252	2.175687
62	2886	1	Hybrids	D	D	D	2886	2	2886_2	-0.738276	1.994280
63	2886	1	Hybrids	D	D	D	2886	3	2886_3	-0.688949	2.094615
64	2886	1	Hybrids	D	D	D	2886	4	2886_4	-1.045022	1.725989
65	2886	1	Hybrids	D	D	D	2886	5	2886_5	-0.874548	2.455010
66	2886	1	Hybrids	D	D	D	2886	6	2886_6	-0.780685	1.973056
67	2886	1	Hybrids	D	D	D	2886	7	2886_7	-0.780872	1.857325

68	2886	1	Hybrids	D	D	D	2886	8	2886_8	-1.115636	1.438587
69	2886	1	Hybrids	D	D	D	2886	9	2886_9	-1.500357	0.870348
70	2886	1	Hybrids	D	D	D	2886	10	2886_10	-1.180198	1.885066
71	2886	2	Haploids	H	H	H	2886	1	2886_1	-1.531912	1.547685
72	2886	2	Haploids	H	H	H	2886	2	2886_2	-1.706477	0.927235
73	2886	2	Haploids	H	H	H	2886	3	2886_3	-1.775090	0.497977
74	2886	2	Haploids	H	H	H	2886	4	2886_4	-1.698461	0.407072
75	2886	2	Haploids	H	H	H	2886	5	2886_5	-1.846853	0.826711
76	2886	2	Haploids	H	H	H	2886	6	2886_6	-1.612049	0.735404
77	2886	2	Haploids	H	H	H	2886	7	2886_7	-1.840642	1.024977
78	2886	2	Haploids	H	H	H	2886	8	2886_8	-1.781602	0.515191
79	2886	2	Haploids	H	H	H	2886	9	2886_9	-1.821878	0.467947
80	2886	2	Haploids	H	H	H	2886	10	2886_10	-1.720805	0.776386
81	2900	1	Hybrids	D	D	D	2900	1	2900_1	-0.626221	-0.543009
82	2900	1	Hybrids	D	D	D	2900	2	2900_2	-0.499354	0.186234
83	2900	1	Hybrids	D	D	D	2900	3	2900_3	-0.750400	0.453736
84	2900	1	Hybrids	D	D	D	2900	4	2900_4	-0.046266	0.718087
85	2900	1	Hybrids	D	D	D	2900	5	2900_5	-0.624231	0.402166
86	2900	1	Hybrids	D	D	D	2900	6	2900_6	-0.808922	-0.641443
87	2900	1	Hybrids	D	D	D	2900	7	2900_7	-1.234842	-1.346057
88	2900	1	Hybrids	D	D	D	2900	8	2900_8	-1.161871	-0.670392
89	2900	1	Hybrids	D	D	D	2900	9	2900_9	-1.160558	-1.432141
90	2900	1	Hybrids	D	D	D	2900	10	2900_10	-1.482696	-0.677702
91	2900	2	Haploids	NA	H	H	2900	1	2900_1	-0.803969	-0.420323
92	2900	2	Haploids	D	D	H	2900	2	2900_2	-0.942244	-0.515623
93	2900	2	Haploids	NA	H	H	2900	3	2900_3	-0.942247	-0.209032
94	2900	2	Haploids	D	D	H	2900	4	2900_4	-0.779953	-0.192233
95	2900	2	Haploids	NA	D	H	2900	5	2900_5	-1.051869	0.346418
96	2900	2	Haploids	NA	D	H	2900	6	2900_6	-0.808992	0.071523
97	2900	2	Haploids	D	H	H	2900	7	2900_7	-1.083154	-1.107237
98	2900	2	Haploids	D	D	H	2900	8	2900_8	-1.060087	-0.928532
99	2900	2	Haploids	H	D	H	2900	9	2900_9	-1.009508	0.026311
100	2900	2	Haploids	D	H	H	2900	10	2900_10	-1.192701	-0.519128
101	2903	1	Hybrids	D	D	D	2903	1	2903_1	-0.572797	-0.805194

102	2903	1	Hybrids	D	D	D	2903	2	2903_2	-0.967925	-1.053082
103	2903	1	Hybrids	D	D	D	2903	3	2903_3	-0.306881	-0.783082
104	2903	1	Hybrids	D	D	D	2903	4	2903_4	-0.524033	-0.992374
105	2903	1	Hybrids	D	D	D	2903	5	2903_5	-0.854500	-0.906322
106	2903	1	Hybrids	D	D	D	2903	6	2903_6	-0.340753	-0.767767
107	2903	1	Hybrids	D	D	D	2903	7	2903_7	-0.485835	-0.565339
108	2903	1	Hybrids	D	D	D	2903	8	2903_8	-0.953837	-1.121078
109	2903	1	Hybrids	D	D	D	2903	9	2903_9	-0.240842	-0.691662
110	2903	1	Hybrids	D	D	D	2903	10	2903_10	-0.899189	-0.831000
111	2903	2	Haploids	H	H	H	2903	1	2903_1	-0.818774	-1.604904
112	2903	2	Haploids	NA	H	H	2903	2	2903_2	-1.841438	0.000000
113	2903	2	Haploids	NA	H	H	2903	3	2903_3	-1.336102	-2.196132
114	2903	2	Haploids	H	H	H	2903	4	2903_4	-1.354738	-2.507392
115	2903	2	Haploids	H	H	H	2903	5	2903_5	-1.204612	-2.565290
116	2903	2	Haploids	H	H	H	2903	6	2903_6	-1.150327	-2.191783
117	2903	2	Haploids	NA	H	H	2903	7	2903_7	-1.228756	-2.037717
118	2903	2	Haploids	NA	H	H	2903	8	2903_8	-1.575267	-2.310437
119	2903	2	Haploids	NA	H	H	2903	9	2903_9	-1.636831	-2.354678
120	2903	2	Haploids	H	H	H	2903	10	2903_10	-1.414258	-1.460412

x2867_54	x2886_54	x2900_54	x2903_54	x2722_sRGB	x2820_sRGB	x2867_sRGB	x2886_sRGB	x2900_sRGB
0.367387	0.649022	-0.994215	-0.600683	0.207319	0.085111	0.532100	0.999981	-0.750085
-0.093132	0.552575	-1.426673	-1.012636	-0.114459	-0.353494	0.088349	0.780220	-1.182736
0.370272	0.820383	-0.591107	-0.536665	0.362045	0.566687	0.519852	0.998644	-0.392823
0.663314	0.788910	-0.837100	-0.371468	0.286376	0.571377	0.599521	1.009093	-0.668197
0.041062	0.290196	-1.773076	-0.818492	-0.341452	-0.601297	0.006897	0.469794	-1.640299
-0.159774	0.038117	-1.679917	-0.938041	-0.105753	-0.427517	0.017921	0.449164	-1.199195
-0.265863	0.373044	-1.533781	-0.904933	-0.043744	-0.117202	-0.062688	0.573731	-0.967775
-0.910647	0.000000	-2.760376	-1.497913	-1.011930	-0.798428	-0.635330	0.000000	-1.924670
0.235816	0.700532	-1.384048	-0.711438	0.043302	0.092152	0.165997	0.791002	-1.131918
-0.142895	1.069594	-1.503262	-0.967543	-0.285902	-0.430760	-0.087627	0.911730	-1.208076
-0.697040	-0.843413	-2.459971	-1.415076	-0.957700	-1.473504	-0.470160	-0.289076	-1.989262
-1.185857	-1.244249	-2.813694	-1.683863	-1.233786	-1.489380	-0.767785	-0.491593	-2.154139
-1.083688	0.000000	-2.743796	-1.653776	-1.206367	-1.258769	-0.808447	0.000000	-2.231555
-0.930017	0.000000	-2.701697	-1.624509	-1.224595	-1.289727	-0.695143	0.000000	-2.300905
-0.678972	0.000000	-2.206331	-1.282026	-0.863097	-1.213453	-0.471767	0.000000	-1.895495
-1.001028	-1.115988	-2.661438	-1.460361	-0.979662	-1.235263	-0.581495	-0.441482	-1.881036
-0.914197	-1.139282	-2.760495	-1.453846	-1.001673	-1.053563	-0.708620	-0.467522	-1.989137
-0.973751	0.000000	-2.856264	-1.626228	-1.115465	-1.002889	-0.654575	0.000000	-2.025625
-1.133620	-0.855087	-2.594207	-1.461821	-1.185795	-1.208612	-0.709427	-0.691578	-1.941361
-1.045833	0.000000	-2.907122	-1.641727	-1.211088	-1.495184	-0.747613	0.000000	-2.417495
-0.172595	0.055024	-1.271801	-0.708726	0.079001	-0.647207	-0.387349	0.251265	-0.715150
-0.345207	-0.504014	-1.905096	-1.012836	-0.152411	-0.917839	-0.624265	-0.206728	-1.138397
-0.059723	-0.342723	-1.710440	-0.714651	0.021739	-0.701815	-0.472744	-0.137778	-1.131613
0.101718	-0.414041	-1.576045	-0.523658	0.054195	-0.418140	-0.529467	-0.162293	-1.157510
-0.630946	-1.022450	-1.883017	-0.854067	-0.478753	-0.943011	-0.913151	-0.520341	-1.313716
-0.569741	-0.526511	-1.791291	-0.855781	-0.032521	-1.087296	-0.683088	-0.288830	-0.914690
-0.329099	-0.223146	-1.698870	-0.868432	0.152210	-0.743379	-0.498360	0.010875	-0.762030
0.278191	0.053475	-1.467046	-0.359574	0.183884	-0.247629	-0.285422	0.062596	-0.816106
-0.044931	-0.247737	-1.869843	-0.728210	0.070665	-0.821186	-0.499430	-0.184004	-1.231591
-0.129579	0.029566	-1.728468	-0.675382	0.139569	-0.762891	-0.432453	-0.142146	-1.111469
-0.165886	-0.742494	-1.522255	-0.761041	-0.043264	-1.285103	-0.487895	-0.246452	-1.041536
-0.719491	-0.647445	-1.843276	-1.068169	-0.273569	-1.514931	-0.658442	-0.165475	-1.247176
-0.144425	-0.294825	-1.468031	-0.642205	0.043841	-0.932618	-0.347824	-0.011976	-0.922021

-0.147111	-0.201337	-1.680704	-0.747320	-0.068409	-0.970633	-0.497519	-0.051189	-1.225146
-0.315088	-0.863026	-1.755448	-0.706369	-0.251540	-1.279026	-0.678246	-0.477263	-1.292381
-0.600129	-0.748485	-1.700606	-0.696589	-0.145444	-1.145704	-0.702914	-0.292110	-0.859453
-1.107871	-0.887216	-1.992620	-1.145953	-0.472512	-1.027030	-1.069875	-0.334818	-1.022449
-0.617588	-0.632101	-1.781173	-0.812063	-0.150038	-0.997809	-0.793952	-0.292540	-0.946255
-0.611680	-0.597031	-2.029911	-0.985740	-0.230778	-0.832983	-0.877843	-0.316960	-1.267310
-0.563191	-0.507126	-1.998641	-0.856050	-0.167549	-0.993510	-0.797302	-0.445767	-1.357111
0.014306	0.180398	-1.553505	-1.015780	-0.276079	-0.436094	-0.117102	0.693983	-1.186374
-0.418677	0.098441	-2.198454	-1.230120	-0.343271	-0.317197	-0.532859	0.517240	-1.623837
-0.342431	0.171613	-2.221582	-1.188260	-0.592693	-0.287160	-0.560106	0.513414	-1.761486
-0.126339	0.257107	-1.883429	-0.999588	-0.408167	0.061461	-0.323247	0.627200	-1.396919
-0.325601	-0.171968	-2.558777	-1.344738	-1.205128	-0.668944	-0.619679	0.270230	-2.160240
-0.589834	-0.265047	-2.394583	-1.316914	-0.387339	-0.677506	-0.612607	0.288823	-1.632818
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