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ORIGINAL ARTICLE

Modeling some mineral nutrient requirements for micropropagated wild apricot shoot cultures

Irina Y. Kovalchuk[1] · Zinat Mukhitdinova[1] · Timur Turdiyev[1] · Gulnara Madiyeva[1] · Melekşen Akin[2,5] · Ecevit Eyduran[3] · Barbara M. Reed[2,4]

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Abstract A response surface methodology (RSM) experimental design was applied for improving micropropagation of a wild apricot, *Prunus armeniaca* Lam., from the mountains of Kazakhstan. In an initial study, woody plant medium (WPM) mineral nutrients [calcium nitrate, ammonium nitrate, mesos (calcium chloride, potassium phosphate and magnesium sulfate) potassium sulfate and minor nutrients] were tested in a response surface methodology (RSM) experiment. Shoot quality was the best when nitrogen and mesos ($CaCl_2$, $MgSO_4$, K_2SO_4, KH_2PO_4) compounds were altered. In this study an expanded mesos optimization experiment was run. Data taken included a subjective quality rating, shoot length, shoot number, leaf color and size, callus and physiological disorders. Data were analyzed by Classification and Regression Tree Analysis (CART), a data mining technique that provides specific cutoff values for data and easy to interpret data trees. The CART analysis indicated that the best quality would be with ≤2.4× WPM levels of KH_2PO_4 and ≤0.75× $MgSO_4$. Shoot length was affected by K_2SO_4, but most shoots were of good size at any concentration. Shoot multiplication was affected by KH_2PO_4, but there were >5 shoots at any concentration. Leaf color was best with ≤2.41× KH_2PO_4 and ≤1.22× K_2SO_4. Based on the CART analysis, a recommendation for improved mesos compounds was developed. Each of the individual trees was analyzed and the cutoff points determined so that all the growth characteristics could be considered in the final concentrations chosen. Using the combined results from the CART analysis, the suggested medium would include WPM with $CaCl_2$ 2.7×, $MgSO_4$ 2.7×, K_2SO_4 0.8×, KH_2PO_4 0.75×.

Keywords CART data mining algorithm · In vitro culture · Mineral nutrition · *Prunus armeniaca* · Response surface methodology

✉ Barbara M. Reed
reedba@onid.oregonstate.edu

Irina Y. Kovalchuk
kovalchuk_i_u@mail.ru

Melekşen Akin
akinmeleksen@gmail.com

Ecevit Eyduran
ecevit.eyduran@gmail.com

[1] Institute of Plant Biology and Biotechnology, Timiryazev Str. 45, 050040 Almaty, Republic of Kazakhstan

[2] Department of Horticulture, Oregon State University, ALS 4017, Corvallis, OR 97331, USA

[3] Biometry Genetics Unit, Department of Animal Science, Agricultural Faculty, Igdir University, Igdir, Turkey

[4] United States Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository, 33447 Peoria Road, Corvallis, OR 97333, USA

[5] Present Address: Department of Horticulture, Agricultural Faculty, Igdir University, Igdir, Turkey

Introduction

Tissue culture medium optimization is a complex process due to the effects and interactions of many factors. Therefore, effective mineral nutrition optimization requires careful experimental design and statistical analysis in order to screen many factors at a range of reasonable levels and determine any interactions. Response surface methodology (RSM) is an effective statistical tool which combines experimental designs and analyses for optimization processes. RSM optimal designs were shown to be a useful tool for tissue culture studies by reducing treatment combinations tremendously compared to traditional factorial designs (Akin 2016; Niedz and Evens 2007; Reed et al. 2013).

Data mining algorithms are also useful for analysis of medium optimization experiments with multiple factors. The Chi-Squared Automatic Interaction Detection (CHAID) data mining algorithm was used previously to model common mineral nutrient requirements for diverse hazelnut genotypes (Akin et al. 2016). CHAID constructs non-binary, easy to interpret visual trees. The algorithm detects both linear and non-linear effects, that are not detectable by traditional statistical techniques like regression and anova (Akin 2016). Neurofuzzy logic was also used to design better micropropagation media by modeling eight ions for three growth parameters pf *Prunus* rootstocks (Alanagh et al. 2014) and predicting plant responses correlated to nutrient levels in a variety of apricot tissue culture media (Gago et al. 2011).

Another option for data analysis that has not been used for plant tissue culture optimization is Classification and Regression tree (CART) data mining algorithm. CART constructs visual, easy to interpret binary decision trees. The algorithm is a non-parametric statistical technique which does not require a normality assumption for the dependent variables. CART performs well in the case of missing data of independent variables, and it can be applied to both large and small data sets. Although the CART algorithm is capable of detecting interactions for continuous, nominal and ordinal variables (Yordanova et al. 2015), it has not been applied for analyzing tissue culture data. The CART modeling procedure consists of tree building and pruning. All the input variables are considered for the best split in the tree building process. The algorithm makes trial splits until the best split is found which minimizes the variance within subsets and maximizes the variance between subsets (Rashidi et al. 2014). The Least Squares Deviation (LSD) method is used for continuous variables as a splitting criterion (SPSS 2013). The CART algorithm performs a backward pruning (stopping) procedure consisting of complete tree building, after which it continues analyzing and pruning back the tree to optimal size. A V-fold cross-validation is used in this procedure to evaluate the overall accuracy of the technique (Rashidi et al. 2014).

The initial RSM study for optimizing shoot growth of a wild apricot, *Prunus armeniaca* Lam, indicated that the nutrients with the greatest impact on shoot growth were the meso compounds ($CaCl_2$, KH_2PO_4, $MgSO_4$ and K_2SO_4) and nitrogen groups (Kovalchuk et al. 2017). Growth of the shoots on a trial medium with 2.5× Mesos and 1.75× nitrogen greatly improved growth but was not optimal (Kovalchuk et al. 2017). The goal of the current study was to further analyze the meso compounds within a RSM optimal design to provide the information required for a finalized growth medium for *P. armeniaca* and possible testing for other apricot species and cultivars.

Materials and methods

Plant material and culture conditions

Seedlings used to initiate cultures were germinated from fruit of a selected tree of the wild apricot, *Prunus armeniaca* Lam., collected from the Zailiyskyi Alatau region of Kazakhstan, a part of the Ketmen metapopulation, Site 2B, Bolshoe Aksu Gorge, Site 2, Tree 7. The tree location was 1595 M, 43° 17.917 min N, 79° 37.954 min E. Shoot cultures were initiated from 10 seedlings derived from seeds of a single 10–15 year old tree. Shoots were pooled to provide a culture representing the genetic diversity present in the original population. Shoots were initially propagated on woody plant medium (WPM) (Lloyd and McCown 1980) and later on a trial medium (Kovalchuk et al. 2017) of WPM salts and vitamins, with increased nitrogen (1.75×) and mesos ($CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, K_2SO_4, and KH_2PO_4) (2.5×), 20 g each sucrose and sorbitol and 0.5 mg/L (2.2 µM) N^6-benzyladenine (BA) (PhytoTechnology Labs, Shawnee Mission, KS), 4 g agar (Bitek, Difco, Detroit, MI.) and 1.5 g Gelrite (PhytoTechnology Labs) at pH 5.7. Shoots were grown in Magenta GA7 boxes with 40 ml of medium in each box with a transfer to fresh medium every 3 weeks. Cultures were grown at 24 °C under a 16-h photoperiod with an average of 40 µM m^2 s^{-1} radiation provided by cool white fluorescent lamps.

Experimental design

A RSM D-optimal experimental design was set up with Design-Expert software (Design-Expert 2010) using $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, K_2SO_4, and KH_2PO_4 salts as independent factors within 27 treatment combinations (Table 1). The $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$, K_2SO_4, and KH_2PO_4 salts were tested in a range of 0.5–3.0× WPM within the design. The other major and minor salts were left at their original WPM concentrations (1×). Shoots were planted on each treatment medium in duplicate magenta GA7 boxes with 5 shoots per box. Some treatments had additional replications built into the design. Cultures were grown for 3 weeks then transferred twice to the same treatment medium for a total of 9 weeks of culture. Culture conditions were the same as described above. Boxes were randomized on the growth room shelf.

Response surface methodology (RSM) optimal design and the software used adds some additional replicates to estimate the lack of fit. RSM statistical analyses show lack of fit when the model fails to determine the relationship

Table 1 Four factor RSM D-optimal design including 27 treatment points

Treatments	$CaCl_2 \cdot 2H_2O$	$MgSO_4 \cdot 7H_2O$	K_2SO_4	KH_2PO_4
1	0.87	0.5	1.77	2.17
2	0.5	0.5	1.94	0.5
3	1.75	1.75	1.75	1.75
4	2.04	0.5	2.81	3
5	0.5	1.41	3	2.09
6	2.05	3	0.5	2.38
7	3	0.5	1.05	1.73
8	0.5	2.06	2	0.74
9	2.09	1.13	0.5	0.5
10	1.75	1.75	1.75	1.75
11	3	3	3	2.44
12	2.05	3	0.5	2.38
13	2.09	1.13	0.5	0.5
14	2.06	0.5	1.92	0.81
15	0.5	2.67	0.5	0.81
16	3	3	1.38	0.5
17	1.9	2.47	2.25	3
18	1.25	3	3	0.5
19	3	1.07	3	0.5
20	0.5	3	1.95	3
21	1.66	1.77	1.77	1.73
22	2.88	2.74	3	0.79
23	3	1.77	1.05	3
24	1.75	1.75	1.75	1.75
25	0.5	0.5	0.5	3
26	1	1	1	1
27	1	1	1	1

Design points 1–25 for investigating the effects of individual factors on mineral nutrition of *Prunus armeniaca* and WPM medium controls (points 26–27). Amounts listed are relative to the standard WPM concentration (×WPM)

between the factors and the response, which indicates that the model is not significant (Anderson and Whitcomb 2005). The CART algorithm used to analyze the data followed the RSM analysis using significant models and therefore did not require an additional estimate of the lack of fit. A V-fold cross-validation criterion was used for evaluating the accuracy of the model (Rashidi et al. 2014).

Data

Three shoots from each of the duplicate culture vessels were evaluated (n = 6), always evaluating plants selected on a diagonal from the box label. The remaining four shoots were photographed to document general appearance. Data evaluation was as follows: shoot quality, a subjective visual assessment of shoot vigor and form was assessed as 1 = poor, 2 = moderate and 3 = good. Shoots longer than 5 mm were counted. The longest shoots were measured in centimeters. Callus formation was evaluated as: 1 = callus ≥2 mm, 2 = callus <2 mm, and 3 = absent (no callus present). Leaf size was rated as: 1 = small leaves, 2 = moderate leaves, 3 = big leaves. Leaf color was evaluated as: 1 = yellow or red, 2 = light green, 3 = green. Several physiological disorders were evaluated: green veins and shoot tip necrosis were rated as 1 = much, 2 = some, 3 = none.

Data structure

The outputs (plant quality, shoot length, shoot multiplication, callus formation, leaf color and size) and inputs ($CaCl_2 \cdot 2H_2O$, $MgSO_4 \cdot 7H_2O$, K_2SO_4, and KH_2PO_4) were considered as continuous variables because the average response per treatment was calculated. Therefore, all the variables were specified as scaled within the IBM SPSS 23 statistical software (SPSS 2013).

Statistical analysis

The CART algorithm was used to predict optimal responses from the independent variables using both continuous and categorical data. A separate regression tree for each response was generated by the algorithm. As a nonparametric statistical technique, CART constructs a binary decision tree by partitioning a node (subset) into two new subsets recursively, until the variance within the subsets is minimized and the variance between the subsets is maximized (Ali et al. 2015; Nisbet et al. 2009). The Pearson correlation coefficient in each dependent variable between actual and predicted values is maximized, which is an indicator of the predictive performance of the algorithm. The hypothesis of the correlation coefficient was tested with a two-sided t-test.

The command order followed in IBM SPSS software (SPSS 2013) was Analyze > Classify > Tree. CART was selected as the method to construct a regression tree diagram because the dependent variables (shoot quality, shoot length, shoot number, callus, leaf color, leaf size, shoot tip necrosis and green vein formation) were scaled. In the construction of the regression tree graph the cross-validation value of 10 was used, as required by the algorithm.

The CART modeling procedure consists of tree building and pruning. Pruning is removing the unnecessary branches of the tree by assigning numbers to the parent and child nodes. The numbers are assigned based on the highest Pearson correlation of the generated trees (Ali et al. 2015; Nisbet et al. 2009). Minimum parent and child node numbers were assigned as 4:2 to obtain the highest Pearson correlation between actual and predicted values for each response. By default, tree depth of the CART algorithm was used as 5. The pruning operation was activated to prevent unnecessary branching for each generated regression tree. All the factors are considered for the best split in the tree building process. The algorithm performs trial splits until the best split is determined by minimizing the variance within groups and maximizing the variance between groups. The Least Squares Deviation (LSD) method is used for continuous variables as a splitting criterion (SPSS 2013). The CART algorithm performs a backward pruning (stopping) procedure consisting of complete tree building, after which it continues analyzing and pruning back the tree to optimal size. A V-fold cross-validation criterion is used for evaluating the accuracy of the model (Rashidi et al. 2014).

Results

Quality

The subjective evaluation of shoots indicated by the quality rating (1 poor, 2 moderate, 3 good) is an overall summation of the other objectively measured responses and is the most useful for determining an improved growth medium (Niedz et al. 2007). A decision tree diagram for plant quality was generated by the CART data mining algorithm (Fig. 1). Three compounds, KH_2PO_4, $MgSO_4{\cdot}7H_2O$ and $CaCl_2{\cdot}2H_2O$, were significant for shoot quality. The Pearson correlation coefficient between actual and predicted values for quality was 0.70 ($p<0.01$), indicating a

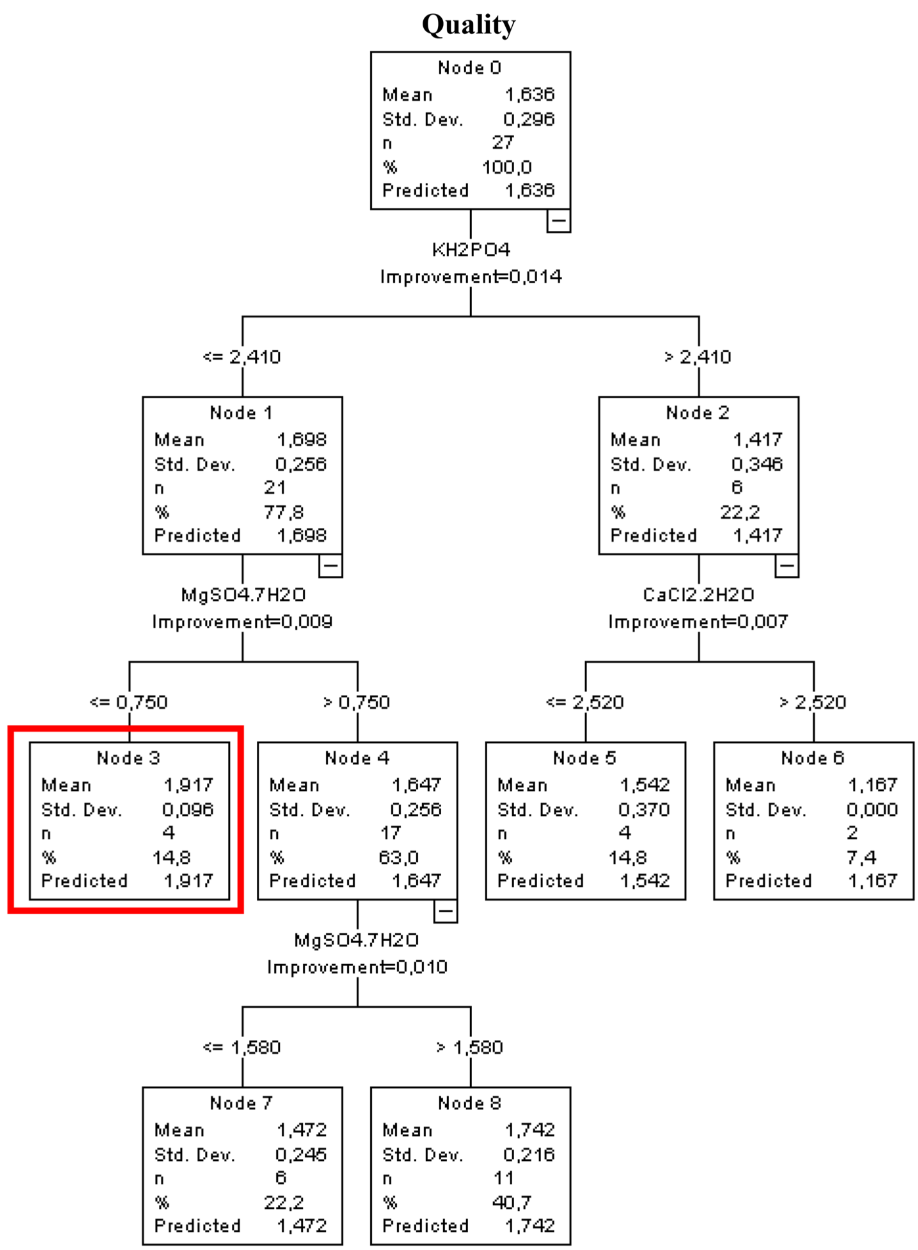

Fig. 1 The CART decision tree diagram for plant quality of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×WPM. Mean and predicted values are based on plant quality ratings of *1* poor, *2* moderate, *3* good given to each shoot

high predictive ability of the algorithm. Predicted average quality was 1.64 ± 0.3. The root node (Node 0) at the top of the regression tree diagram was partitioned into two child subsets (Nodes 1 and 2) based on KH_2PO_4 concentration. Node 1, consisting of 21 treatment combinations (n = 21), was a subset of the plants exposed to 2.41× or less KH_2PO_4 with predicted average plant quality of 1.7. Node 2 (n = 6) was a subset of shoots exposed to KH_2PO_4 >2.41× with predicted average shoot quality of 1.42. Node 1 was split into Nodes 3 and 4 according to $MgSO_4·7H_2O$. Node 3 (n = 4) was a terminal subset with the highest plant quality (1.92 ± 0.1), and was within the concentration ranges of KH_2PO_4 ≤2.41× and $MgSO_4·7H_2O$ ≤0.75×. Node 4 (n = 17) was the subset of shoots exposed to KH_2PO_4 ≤2.41× and $MgSO_4·7H_2O$ >0.75×, and divided into two child subsets by $MgSO_4·7H_2O$ with lower predicted quality ratings (Fig. 1). Node 8 was a subset of Node 4 with $MgSO_4·7H_2O$ >1.58 and a quality rating of 1.74 ± 0.22, similar to that of Node 3.

Shoot length and multiplication

K_2SO_4 was the only significant factor affecting shoot length (Fig. 2). The Pearson correlation coefficient between actual and predicted shoot length was low at 0.4 (p < 0.05). Node 1 contained the longest shoots (10.2 cm) and required K_2SO_4 ≤1.85×. Multiplication was only affected by KH_2PO_4 (Fig. 3). The Pearson correlation coefficient between actual and predicted shoot number was 0.64 (p < 0.01). The highest predicted shoot number (average of 9.4) was obtained in Node 3 and required a concentration range of 0.77× <KH_2PO_4 ≤0.91×. However the number of shoots per plant ranged from 5 to 9 and this indicates that all KH_2PO_4 concentrations tested would provide good multiplication.

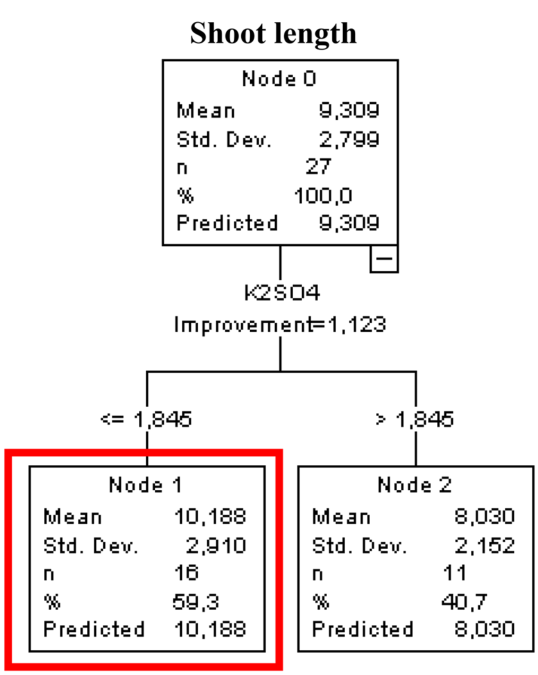

Fig. 2 The CART decision tree diagram for shoot length (cm) of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×WPM

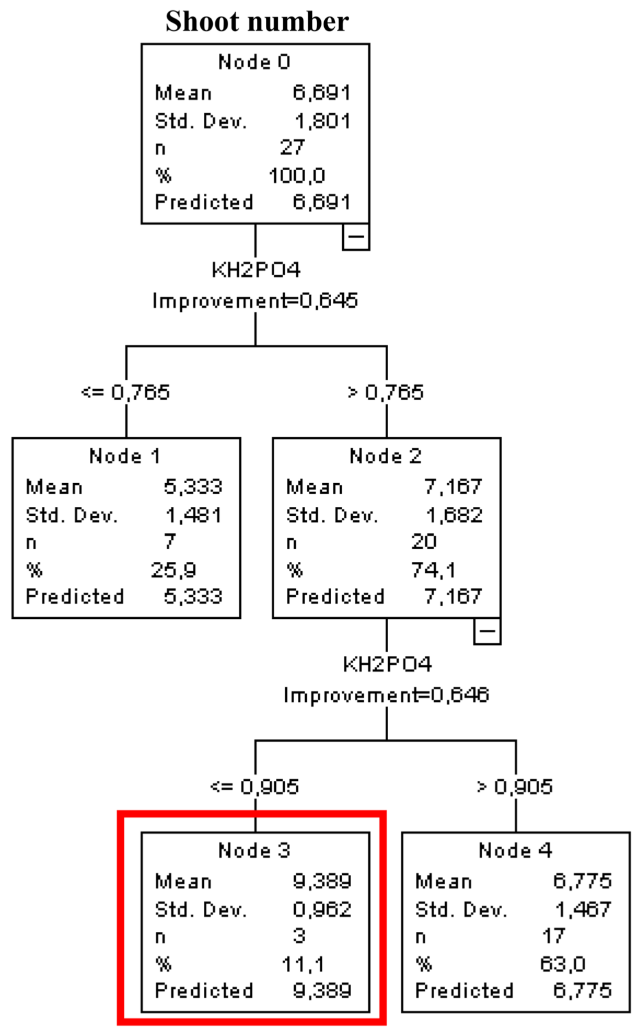

Fig. 3 The CART decision tree diagram for shoot number of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×WPM

Callus

Callus formation on the shoots was significantly affected by $MgSO_4·7H_2O$ and K_2SO_4 (Fig. 4). The Pearson correlation coefficient between actual and predicted callus formation was 0.80 (p < 0.01), inferring high predictive ability for this response. The most callus formation (mean rating of 2) was recorded in Node 1, which was exposed to $MgSO_4·7H_2O$ ≤2.57× (Fig. 4). The least callus formation (rating of 3) was obtained in Node 3, which required concentration

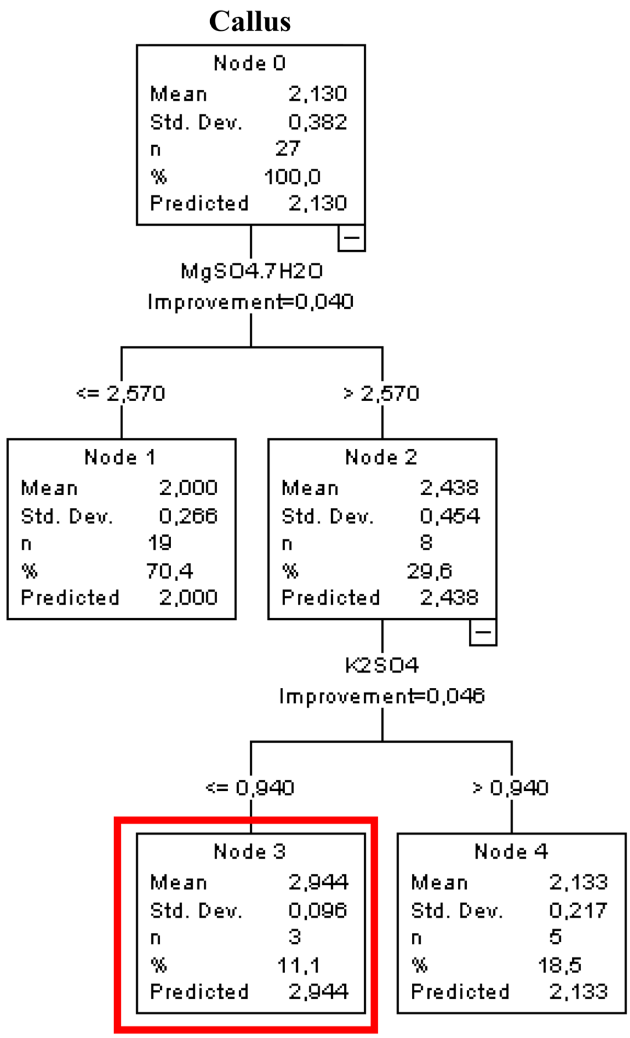

Fig. 4 The CART decision tree diagram for callus formation of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×DKW. Mean and predicted values are based on the rating *1* large callus, *2* moderate callus, *3* no callus given to each shoot

ranges of $MgSO_4{\cdot}7H_2O$ >2.57× and K_2SO_4 <0.94, indicating an interaction between these two factors.

Leaf color and leaf size

Leaf color was affected by KH_2PO_4, K_2SO_4 and $CaCl_2{\cdot}2H_2O$ (Fig. 5). The Pearson correlation coefficient between actual and predicted values for leaf color was 0.74 ($p<0.01$). The 27 treatment combinations were divided in two subgroups based on 2.41× KH_2PO_4 as the critical concentration threshold. Of these, 21 treatment combinations were assigned to Node 1 and six to Node 2. The shoots in Node 3 had the greenest leaves and were within the concentration ranges of KH_2PO_4 ≤2.41× and K_2SO_4 ≤1.22×. The shoots with palest leaves were in Node 6 (mean rating of 1)

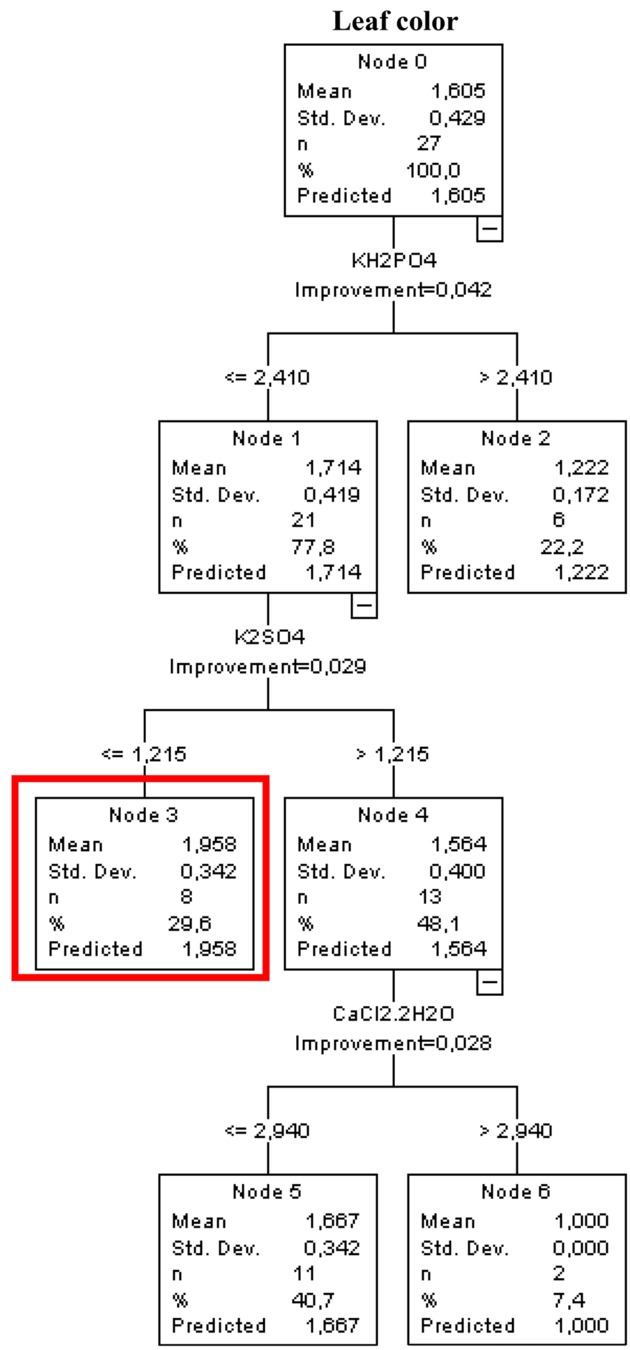

Fig. 5 The CART decision tree diagram for leaf color of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×WPM. Mean and predicted values are based on the rating *1* yellow or red, *2* light green, *3* green to each shoot

and were within the concentration levels of K_2SO_4 >1.22× and $CaCl_2{\cdot}2H_2O$ >2.94×.

Leaf size was influenced by KH_2PO_4 and $CaCl_2{\cdot}2H_2O$ (Fig. 6). Most of the shoots (Node 2) had very small leaves when grown on KH_2PO_4 >0.77. The largest leaves (Node

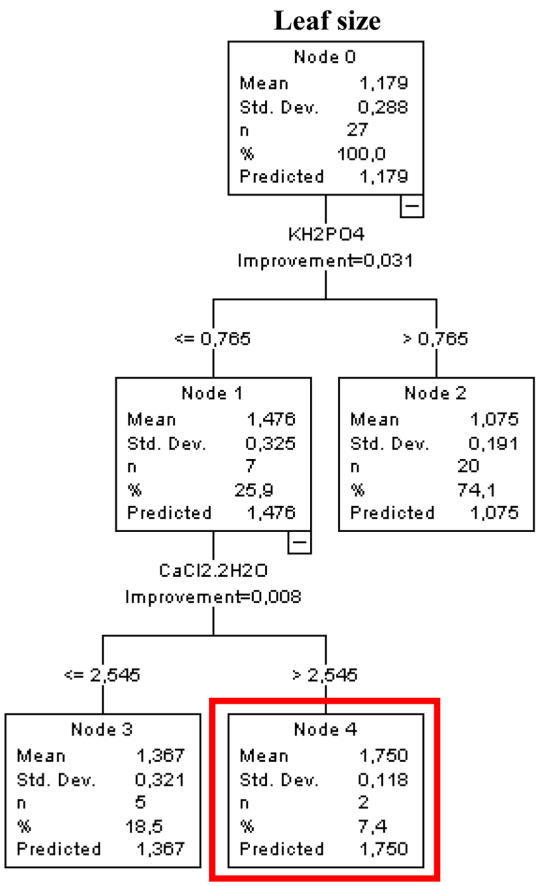

Fig. 6 The CART decision tree diagram for leaf size of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×WPM. Mean and predicted values are based on the rating *1* small leaves, *2* moderate leaves, *3* big leaves given to each shoot

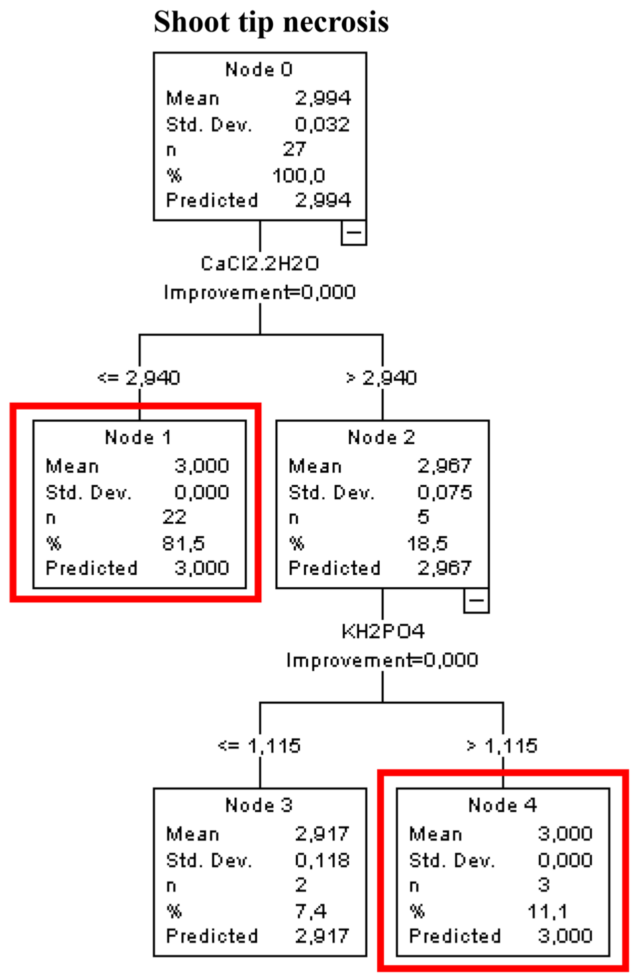

Fig. 7 The CART decision tree diagram for shoot tip necrosis of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×WPM. Mean and predicted values are based on the rating: *1* much, *2* some, *3* none shoot tip necrosis formation

4) were still moderate to small (rating 1.75) based on KH_2PO_4 ≤0.77 and $CaCl_2·2H_2O$ >2.55.

Physiological disorders

Very little shoot tip necrosis (STN) was seen in the current study, however STN was affected by 2.94× $CaCl_2·2H_2O$ as the critical threshold concentration (Fig. 7). With $CaCl_2·2H_2O$ ≤2.94× no STN was predicted. Another range with no STN formation was when $CaCl_2·2H_2O$ >2.94×, but within this range there was an interaction with KH_2PO_4, and the requirement for KH_2PO_4 was above 1.12×. The Pearson correlation coefficient between observed and predicted shoot tip necrosis formation was 0.70 ($p<0.01$).

The development of green veins noted in the study was affected by KH_2PO_4 and $CaCl_2·2H_2O$ (Fig. 8). The least green vein formation (rating 2.95) was predicted for KH_2PO_4 >0.62× (Node 2). Node 4 showed the most green vein formation (rating 1.83) when KH_2PO_4 ≤0.62× and $CaCl_2·2H_2O$ >2.55×. The Pearson correlation coefficient between real and predicted green vein formation was 0.70 ($p<0.01$).

Discussion

Advanced experimental design and statistical analysis methods provide more precise ways of looking at the problem of mineral nutrient optimization. Optimizing mineral nutrients in growth medium using RSM experimental design provides an important tool for improving plant growth in tissue culture. The successful optimization of pear, hazelnut and raspberry shoot cultures using RSM experimental design and analysis showed that RSM was a logical choice for determining the best nutrients for improving the growth of Kazakhstan's native apricot *P.*

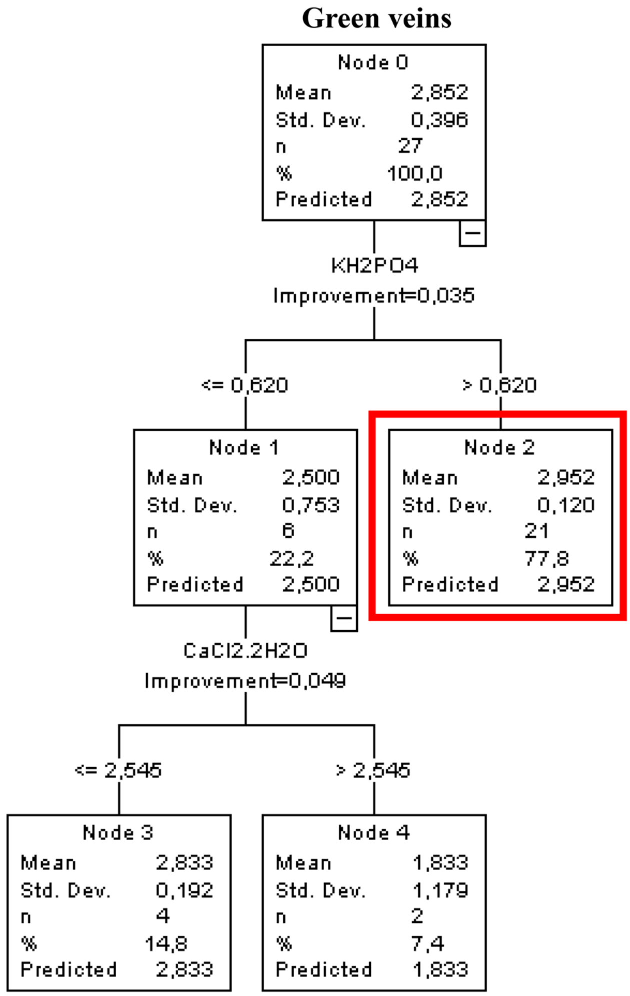

Fig. 8 The CART decision tree diagram for green vein formation of *Prunus armeniaca* apricot. Nodes were determined by the significance of the factors. Salt cut-off values are ×WPM. Mean and predicted values are based on the rating: *1* much, *2* some, *3* none green vein formation

armeniaca (Hand et al. 2014; Poothong and Reed 2014; Reed et al. 2013). The effects of nutrients on shoot growth could be determined through analysis of related groups of nutrients (Reed et al. 2013; Wada et al. 2015). The initial screening of *P. armeniaca* testing five WPM salt groups was used to determine the key nutrients driving apricot shoot growth (Kovalchuk et al. 2017). The poor growth on WPM (Fig. 9a) was improved and that study produced a trial modified WPM with increased nitrogen and mesos (1.75× NH_4NO_3 and $Ca(NO_3)_2$, and 2.5× mesos) that produced much taller shoots with good multiplication, but had poor leaf color and leaf shape with a large callus at the base (Fig. 9c). Continued optimization of the medium by testing the individual mesos components was a needed step.

RSM analysis provided guidance in selecting the most important changes needed in growth medium nutrients, however the program did not distinguish clear cutoff points to use when formulating a new medium (Kovalchuk et al. 2017). The CART algorithm clarified the analysis by RSM because it provided clear division points indicating superior or inferior plant growth based on specific nutrient concentrations (Figs. 1, 2, 3, 4, 5, 6, 7, 8). Another form of data mining is neuro-fuzzy logic that was used to analyze a group of medium experiments with apricot cultivars (Gago et al. 2011). The process indicated that the most important interaction was cultivar × BA for shoot number and productivity, while cultivar × NH_4^+ and BA × magnesium were also important (Gago et al. 2011). In the current study BA was 2.2 µM and not varied, however it is in the same range of good growth seen by Gago et al. (2011). Low BA × low Mg^{2+} was most productive (shoot no × shoot length) for the cultivars studied by Gago et al. and in the current study $MgSO_4$ ≤0.75× or ≥1.6× was significant for overall shoot quality but was not related to shoot length or shoot number with 2.2 µM BA (Figs. 1, 2, 3). Gago et al. also noted that a high Ca^{2+} concentration was favorable for shoot length, however in the current study $CaCl_2$ at >2.6× produced the largest leaf size and at ≤2.9× decreased STN but did not affect shoot length (Figs. 6, 7). Because $Ca(NO_3)_2$ is the nitrogen source in WPM, the amount of calcium is also dependent on the nitrogen level. The $Ca(NO_3)_2$ required for improved growth in the original study (Kovalchuk et al. 2017) was 1.75× that of WPM, so this would also increase the Ca in the medium. A follow-up study on nitrogen requirements is in progress. High $CaCl_2$ combined with very low (<0.6×) KH_2PO_4 promoted the physiological disorder of dark green leaf veins (Figs. 8, 9d).

Comparison of established growth media, slight adjustments to those media, or changes in growth regulators are the most common growth medium experiments. Apricots are considered difficult to micropropagate and numerous studies have attempted to improve the growth medium for a range of cultivars (Marino et al. 1993; Pérez-Tornero and Burgos 2000, 2007). Q&L medium (Quoirin and Lepoivre 1977) is commonly used for apricots, as are MS (Murashige and Skoog 1962), DKW (Driver and Kuniyuki 1984) and the WPM used in the current study. A comparison of six medium formulations showed that shoot number, length and productivity of four genotypes were affected by both genotype and medium (Pérez-Tornero and Burgos 2000). Medium type alone was not significant for shoot length although there were genotype × medium interactions, however BA concentration was highly significant (Pérez-Tornero and Burgos 2000). Deficiency symptoms were noted with the use of WPM for apricot due to the low salt content of the medium. Q&L medium with changes to the growth regulators was suggested as a general apricot medium (Pérez-Tornero and Burgos 2007). These experiments with cultivars compared commonly used existing

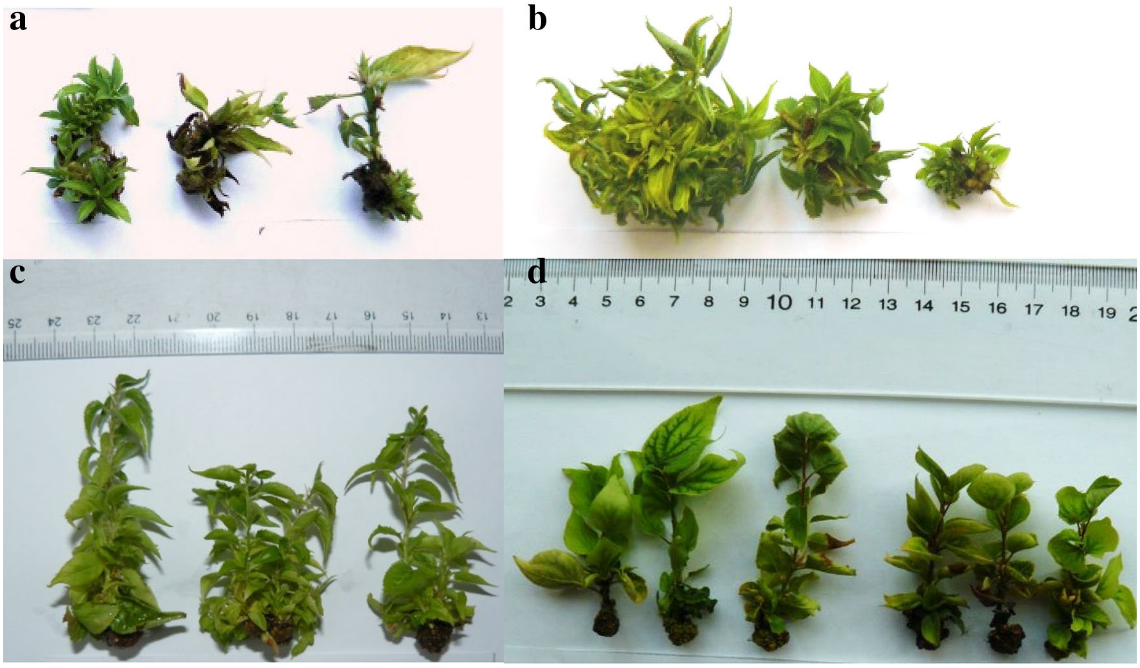

Fig. 9 Growth of *Prunus armeniaca* on **a** control woody plant medium. **b** Mesos treatment 15. **c** The trial medium developed from the original screen (Kovalchuk et al. 2017) and **d** treatment showing callus and green vein symptoms

growth media to determine one that would work for the cultivars in question, but did not directly determine which nutrients were the most important for improved shoot growth.

In the current mesos study, shoots grown on the control WPM were very short and had callus formation, small leaves with leaf and shoot tip necrosis (Fig. 9a). There were some clear differences among meso nutrient treatments. The better mesos treatments produced better leaf color and size as well as more shoot multiplication (Fig. 9b). Multiplication was not difficult for this wild apricot, however slightly lower (≤0.9×) KH_2PO_4 concentrations increased the number of shoots per plant from 6.7 to 9.4. Shoot length increases were nominal, but were improved slightly when K_2SO_4 ≤1.9×. Because the nitrogen compounds were not increased in this trial, none of the treatments tested provided overall high-quality shoots. Earlier studies of four diverse apricot cultivars grown on six growth media found some improved growth, but there were no significant differences in shoot length for any cultivars and only two differed for shoot multiplication (Pérez-Tornero and Burgos 2000). The study concluded that many of the media tested had too much ammonium or a poor ratio of ammonium to nitrate or were deficient in some other nutrients, but the exact amounts of excess or deficiency were not determined in that study (Pérez-Tornero and Burgos 2000).

Using advanced statistical methods allows analysis of the individual salts involved in growth media, and should produce a more accurate reformulation of the growth medium than testing available medium formulations. The current CART analysis indicated that 2.7× WPM concentrations of $CaCl_2·2H_2O$ and $MgSO_4·7H_2O$, in combination with lower amounts of KH_2PO_4 (0.75×) and K_2SO_4 (0.8×) were required to improve leaf size and reduce callus and green veins (Table 2; Figs. 4, 6). The initial study with five nutrient factors indicated that callus increased as the mesos components increased (Kovalchuk et al. 2017). The current study also revealed that an interaction of $MgSO_4·7H_2O$ and K_2SO_4 affected callus growth

Table 2 Meso salts optimal-concentration ranges for each shoot characteristic as predicted by CART algorithm for *Prunus armeniaca*

	$CaCl_2·2H_2O$	$MgSO_4·7H_2O$	K_2SO_4	KH_2PO_4
Quality		≤0.75 or >1.58		≤2.41
Shoot length			Any	
Shoot number				Any
Callus		>2.57	≤0.94	
Leaf color			≤1.215	≤2.41
Leaf size	>2.545			≤0.765
Shoot tip necrosis	Any			Any
Green veins				>0.62
Best medium	2.7	2.7	0.8	0.75

Amounts listed are relative to the standard WPM concentration (×WPM)

Table 3 The mesos mineral nutrients of five media. MS (Murashige and Skoog 1962), WPM (Lloyd and McCown 1980), DKW (Driver and Kuniyuki 1984), Q&L (Quoirin and Lepoivre 1977) and the proposed formulation

Nutrients (mg L^{-1})	MS	WPM	DKW	Q&L	New formulation (mg L^{-1})
$CaCl_2 \cdot 2H_2O$	440	96	147	–	259.7 (2.7× WPM)
$MgSO_4 \cdot 7H_2O$	370	370	740	360	999 (2.7× WPM)
KH_2PO_4	170	170	259	270	127.5 (0.75× WPM)
K_2SO_4	–	990	1560	–	792 (0.80× WPM)

such that high levels of $MgSO_4 \cdot 7H_2O$ (>2.57×) and low K_2SO_4 (<0.94) reduced callus formation. This response is quite different from callus formation in hazelnut that was linked to low NH_4NO_3 concentrations (Hand et al. 2014). Shoot length (KH_2PO_4) and shoot number (K_2SO_4) did not change much regardless of the principal salt concentration (Figs. 2, 3) and this would be expected, as the nitrogen compounds tend to heavily influence length and multiplication of woody plants. Meso mineral nutrients of several culture media commonly used for apricots are quite different from this new formulation determined to be optimum with the CART analysis (Table 3).

Although several physiological disorders are often tied to low calcium or magnesium concentrations, the only STN noted in this study was with high $CaCl_2 \cdot 2H_2O$ (>2.94×) or in an interaction with KH_2PO_4 (Fig. 7). Hyperhydricity was not evident in this study although it is considered a major disorder of apricot shoot cultures (Pérez-Tornero and Burgos 2007). Dark green leaf veins, a typical symptom of magnesium deficiency, was commonly seen (Fig. 9d) and could be tied to very low KH_2PO_4 and high $CaCl_2 \cdot 2H_2O$ concentrations in the medium (Fig. 8). Both potassium and calcium in high concentrations can compete with magnesium uptake in plants that can result in reduced amounts of magnesium available to the shoots (Lopez-Lefebre et al. 2001). In raspberry shoot cultures the main nutrient involved in leaf color was magnesium, but $CaCl_2 \cdot 2H_2O$ at double the normal MS medium concentrations improved leaf color (Poothong and Reed 2014).

Conclusions

The CART data mining algorithm was very effective for determining the effect of mesos compounds on shoot growth (Table 2). Based on the CART analysis, improved mesos nutrient concentrations were determined. Each of the individual trees were analyzed and the cutoff points determined so that all the important plant growth characteristics could be considered in the final concentrations chosen.

CART analysis indicated that the best shoot quality would be with ≤2.4× WPM levels of KH_2PO_4 and ≤0.8× $MgSO_4$. Shoot length was affected by K_2SO_4, but most shoots were of good size at any concentration. Shoot multiplication was only affected by KH_2PO_4, but there were >5 shoots at any concentration. Leaf color was best with ≤2.4× KH_2PO_4 and ≤1.2× K_2SO_4, while the largest leaf size was at ≤0.8× KH_2PO_4 and >2.6× $CaCl_2$. A physiological disorder, dark-green leaf veins appeared when KH_2PO_4 was <0.6× and $CaCl_2$ >2.6×, but this was alleviated with increased KH_2PO_4 or reduced $CaCl_2$. Callus production was significantly decreased with $MgSO_4$ >2.6× and K_2SO_4 <0.9×. Using the combined results from the CART analysis, the suggested medium would include WPM with $CaCl_2$ 2.7×, $MgSO_4$ 2.7×, K_2SO_4 0.8×, KH_2PO_4 0.8× (Table 3). This data combined with an analysis of nitrogen compounds will provide an optimized medium for improved growth of this wild apricot.

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References

Akin M (2016) Statistical methods for tissue culture medium optimization and a multiplexed fingerprinting set for hazelnuts. Dissertation, Oregon State University, Corvallis

Akin M, Eyduran E, Reed BM (2016) Use of RSM and CHAID data mining algorithm for predicting mineral nutrition of hazelnut. Plant Cell Tiss Organ Cult. doi: 10.1007/s11240-016-1110-6

Alanagh E, Garoosi GA, Haddad R, Maleki S, Landín M, Gallego P (2014) Design of tissue culture media for efficient *Prunus* rootstock micropropagation using artificial intelligence models. Plant Cell Tiss Organ Cult 117(3):349–359. doi:10.1007/s11240-014-0444-1

Ali M, Eyduran E, Tariq MM, Tirink C, Abbas F, Bajwa MA, Baloch MH, Nizamani AH, Waheed A, Awan MA, Shah SH, Ahmad Z, Jan S (2015) Comparison of artificial neural network and decision tree algorithms used for predicting live weight at post weaning period from some biometrical characteristics in Harnai sheep. Pak J Zool 47(6):1579–1585

Anderson MJ, Whitcomb PJ (2005) RSM simplified: optimizing processes using response surface methods for design of experiments. Productivity Press, New York

Design-Expert (2010) Stat-Ease, Inc., Minneapolis, MN

Driver JA, Kuniyuki AH (1984) In vitro propagation of Paradox walnut rootstock. HortScience 19:507–509

Gago J, Perez-Tornero O, Landin M, Burgos L, Gallego PP (2011) Improving knowledge of plant tissue culture and media formulationby neurofuzzy logic: a practical case of data mining using apricot databases. J Plant Physiol 168(15):1858–1865

Hand CR, Maki S, Reed BM (2014) Modeling optimal mineral nutrition for hazelnut (*Corylus avellana*) micropropagation. Plant Cell Tiss Organ Cult 119:411–425. doi:10.1007/s11240-014-0544-y

Kovalchuk I, Mukhitdinova Z, Turdiyev T, Reed B (2017) Optimization of WPM for a wild Kazakhstan Apricot, *Prunus armeniaca*. Acta Hortic (in press)

Lloyd G, McCown B (1980) Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Comb Proc Int Plant Prop Soc 30:421–427

Lopez-Lefebre LR, Rivero RM, Garcia PC, Sanchez E, Ruiz JM, Romero L (2001) Effect of calcium on mineral nutrient uptake and growth of tobacco. J Sci Food Agric 81:1334–1338

Marino G, Bertazza G, Magnanini E, Altan AD (1993) Comparative effects of sorbitol and sucrose as main carbon energy sources in micropropagation of apricot. Plant Cell Tiss Organ Cult 34(3):235–244. doi:10.1007/bf00029712

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 15:473–497

Niedz RP, Evens TJ (2007) Regulating plant tissue growth by mineral nutrition. In Vitro Cell Dev Biol-Plant 43:370–381.

Niedz RP, Hyndman SE, Evens TJ (2007) Using a gestalt to measure the quality of in vitro responses. Sci Hortic 112(3):349–359

Nisbet R, Elder J, Miner G (2009) Handbook of statistical analysis and data mining applications. Academic Press, London

Pérez-Tornero O, Burgos L (2000) Different media requirements for micropropagation of apricot cultivars. Plant Cell Tiss Organ Cult 63:133–141

Pérez-Tornero O, Burgos L (2007) Apricot micropropagation. In: Jain SM, Häggman H (eds) Protocols for micropropagation of woody trees and fruits. Springer Netherlands, Dordrecht, pp 267–278

Poothong S, Reed BM (2014) Modeling the effects of mineral nutrition for improving growth and development of micropropagated red raspberries. Sci Hortic 165(0):132–141

Quoirin M, Lepoivre P (1977) Improved media for in vitro culture of *Prunus* sp. Acta Hortic 78:437–442

Rashidi S, Ranjitkar P, Hadas Y (2014) Modeling bus dwell time with decision tree-based methods. Transp Res Rec: J Trans Res Board 2418:74–83

Reed BM, Wada S, DeNoma J, Niedz RP (2013) Improving *in vitro* mineral nutrition for diverse pear germplasm. In Vitro Cell Dev Biol-Plant 49:343–355

SPSS (2013) Statistics for Windows, Version 22.0. IBM Corp, Armonk, NY

Wada S, Niedz RP, Reed BA (2015) Determining nitrate and ammonium requirements for optimal *in vitro* response of diverse pear species. In Vitro Cell Dev Biol-Plant 51:19–27

Yordanova A, Gocheva-Illieva S, Kulina H, Yordanova L, Marinov I (2015) Classification and regression tree analysis in modeling the milk yield and conformation traits for Holstein cows in Bulgaria. J Animal Sci Technol 7(2):208–213