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Genotype – environment interaction in tissue cultures of birch

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Summary. In vitro culture experiments were carried out with three birch genotypes characterized by certain genealogical relationships which serve as indicators of genetic similarity or dissimilarity. Each genotype was grown in each of six different environments (medium types), and callus growth and colour were observed. The aim was to improve our understanding of the operation of genetic and environmental effects at the early stages of regeneration in vitro. For this purpose we tried to answer the question as to whether genetic differences exert effects that are consistently distinguishable under different environments or whether environmental differences exert effects that are consistently distinguishable between different genotypes. Since conventional analytical methods, such as the analysis of variance, are inappropriate for providing satisfactory answers to this question, we applied a new concept of interpretation. With the help of this concept we obtained the following results which appear to be unique among their kind. 1) For both characters, callus growth and callus colour, genetic differences are masked only slightly by the environments while environmental differences are almost completely masked by the genotypes. Thus, in the present experiment, interaction is one-sided in the sense that environmental effects interact strongly with genotypic effects but genotypic effects interact only slightly with the environmental ones. 2) Nuclear effects seem to be responsible for the differences observed in callus growth, while the differences in callus colour can be explained by the joint action of nuclear and extranuclear effects.

Key words: Genotype – Environment interaction – Tissue culture – Birch

Introduction

This paper deals with tissue culture experiments on birch calli. The general aim of these experiments is to provide more specific and qualitative information on the effects genotype and environment may have in the formation of the phenotype than can be gained from such classical statistical analyses as the analysis of variance, regression analyses or heritability estimates. However, it is not our purpose to compare these methods with those to be applied here, nor is this possible in all respects since we shall be concerned with answering the following questions:

a) Are there characteristic and consistent differences between genotypes when grown in a clearly defined range of environments?
b) Can certain environments have effects that are consistently different over a specified set of genotypes?

Depending on which of these two questions receives an affirmative answer, one may infer whether the genotype or the environment produces the more discriminating effect or whether both effects are of equal significance. This approach includes the possibility of detecting one-sided effects, such as genotypes producing consistently different responses over the set of environments, but with one of these genotypes reacting identically to different environments. Hence, such a genotype would show homeostasis over a certain environmental range but would be different from all other genotypes. The same could, of course, happen in the reverse direction, such that each of the environments has a different effect on a genotype – with this holding for all genotypes – but in one of these environments two genotypes react identically. Clearly, these aspects cannot be treated by means of variance or

regression analyses. Such one-sided effects play an important role in the regeneration of tissue cultures, in practical breeding and in adaptation of populations. Moreover, the detection of these effects would help to improve our understanding of the significance of genetic versus environmental components in the determination of phenotypic variation. Such problems are particularly relevant in tissue culture if different genotypes of the same species react very differently to the same series of culture conditions. This would have a decisive effect on the search for "optimal" culture conditions. On the other hand, the existence of optimal conditions can be verified only if *all* genotypes show superior reactions under one of the culture conditions applied. This corresponds exactly to the points a) and b), mentioned above. The details of the concept on which these ideas are based were recently developed by Gregorius and Namkoong (1985).

Tissue culture experiments seem to be very suitable for examining such questions because these experiments allow for rather effective control not only of the environmental factors but also of genotypes. Callus cultures are identical copies of the initial genotype and can be brought to nearly the same ontogenetic state. The last fact is of some interest because the reaction of an individual to a certain environmental condition will depend on its respective state. Hence, it is of some importance to investigate genotypes at, as nearly as possible, the same ontogenetic state.

This paper tries to examine particular aspects of the genotype – environment relationship with the help of three genotypes cultivated in six different environmental conditions (medium types). The three genotypes were selected according to genealogical criteria such that each pair of genotypes has a different coefficient of kinship, both with respect to nuclear and extranuclear genetic information. The reaction of the genotypes is demonstrated by two characters, callus growth and callus colour. Growth is likely to be under predominantly nuclear control while colour involves effects of extranuclear genes to some degree.

The results of the experiment suggest that the genetic effects are more discriminatory than the environmental effects and that this is more pronounced in callus colour than in callus growth. This indicates that, for the purposes of tissue culture, the selection of genotypes may, at least in some cases, be of equal or greater significance than variations in environmental conditions.

Methods of interpretation

In order to extract as much information as possible from our experimental results, we will not apply any of the common methods of the analysis of variance. Instead we will employ a concept of the cause – effect-principle suggested by Gregorius (1977) and recently generalized and elaborated in more detail by Gregorius and Namkoong (1985), which puts particular emphasis on genotype – environment interaction. This concept is not subject to the criticism of the analysis of variance so succinctly elaborated by Lewontin (1974), and it furthermore incorporates the recommendations made by the same author (in Suzuki et al. 1981). Since the concept does not yet seem to be widely applied, its elements shall be briefly introduced. However, the graphical representations given later may be sufficiently clear to allow the reader to skip the present section.

The basis of the concept is characterized by the term "distinguishability of genotypic and environmental effects", where distinguishability is described with the help of the genotypic and environmental response functions. The response function of a particular genotype, or its norm of reaction, specifies the reaction of individuals, all having the same genotype, to different environmental conditions for a given trait variable. Similarly, the response function of (or to) a particular environmental situation specifies the reactions of individuals having different genotypes to this environmental situation. Of course this requires specification of the set of genotypes and the set of environments to be considered, and all results obtained are valid for these sets only. Thus, if two genotypes differ completely in their norms of reaction, their effects are said to be consistently distinguishable over all environments. Otherwise, if they have identical norms, their effects are also identical. If either of these situations holds for a particular genotype relative to all of the remaining genotypes under consideration, the effect of this genotype is called *consistent.* If consistency is realized for *all* genotypes, then we speak of *separability of the genotypic effects.* Consistency and separability of environmental effects is analogously defined by considering the environmental response functions in place of the genotypic response functions (norms of reaction). Hence, one-sided separability can occur in the sense that genotypic effects are separable but environmental effects are not, and vice versa. Our experimental results will show that the detection of such situations yields interesting insights which cannot be obtained by application of methods of the analysis of variance.

Since the concept relies on the notion of distinguishability of measurements, and since, as a rule, measurements contain statistical errors (experimental conditions are almost never identically reproducible, and the measurement itself may a priori be of limited precision) a clarifying remark is in order: distinguishability or identity in responses can only be evaluated within the limits of precision set by the scale of measurement and

statistical errors; in this sense identity is the absence of distinguishability. Moreover, this also implies statistical measurement of responses such as averages or relative frequencies.

In the case of quantitative, real-valued (metrical) traits, which are of relevance for the present experiment, consistency of effects is more appropriately defined by the absence of changes in ranking of the response function (Gregorius and Namkoong 1985). That is to say, consistency in the effects of two genotypes, for example, is realized if one of these genotypes shows a higher response than the other for all environmental conditions under consideration or if both genotypes are identical in their responses. In the same manner, two environmental conditions are consistent in their effects if all of the genotypes considered show a higher measure in one of the two conditions or if they are identical under both conditions. If any of the effects are not consistent, i.e. if changes in ranking of response functions occur, the term *interaction* applies, and in this sense the term complies fully with its intuitive background. Moreover, its applicability is not restricted to deviations from linear relationships, neither among the genotypic nor among the environmental response functions, as it is in the analysis of variance. Interaction may be one-sided if, for example, the environmental effects are separable but the genotypic effects are not, so that only the latter would show interaction. Thus, one-sided interaction and one-sided separability are synonomous terms. Again, one-sided interaction or one-sided separability cannot be detected by means of the conventional analysis of variance (for further details, Gregorius and Namkoong 1985).

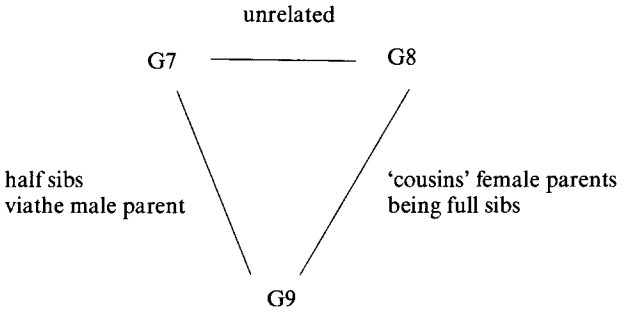
These ideas and notations will form the methodological basis for interpretation of our present experimental results.

The experiment and its representation

Material

Callus cultures were prepared for three genotypes of birch (*Betula pendula*) denoted by G7, G8, G9. These three individuals are characterized by particular genealogical relationships: G7 and G9 are half sibs, the common pollen parent of which is a red birch (*Betula pendula purpurea*) from the Arboretum in Göttingen. G8 and G9 have no common parent, but their female parents are full sibs. In the human population their relationship would be termed 'cousins'. The pollen donor of G8 is a green birch (*Betula pendula tristis*) likewise from the Arboretum. Between G7 and G8 no relationship is known.

Thus the three individuals show pairwise different degrees of kinship and hence different expected degrees of genetic resemblance (consult the following figure). Each of the genotypes was grown in the same range of environments, and the object is to find out whether their reactions to these environments reflect their degrees of resemblance.



Methods

From these three genotypes, callus cultures were prepared and cultivated under experimental conditions, the details of which have been described earlier (Glock and Gregorius 1984). The experiments were carried out as follows: The tissue samples were taken simultaneously from trees of the same age. Furthermore, all cultures experienced the same number of transplantations, so that their ontogenetical states can be considered uniform. All three genotypes with 48 replicates each were exposed to identical environments, with two factors being tested at several levels. In the following explanations only one environmental factor (medium type) with six levels (M1, ..., M6) will be considered. The total experiment also included varying light conditions, namely red light, blue light, white light and dark. However, since the light conditions were identically applied to all medium types, the variation in the latter can be considered as a separate environmental factor, whose influence on performance can be unambiguously studied. In our present study the effects of the different medium types will be of concern. We applied three different medium types, two modified Murashige-Skoog media (M1 to M4, Murashige and Skoog 1962; Srivastava and Steinhauer 1982) and a Linsmayer-Bednar-Skoog medium (M5 and M6) distributed by Boehringer. Sucrose was added to M1, M3 and M5; glucose plus starch was added to M2, M4 and M6.

Two characters were observed, namely callus growth and callus colour. *Callus growth* was measured as volume increase related to the initial volume, so that each growth measurement represents the calculated multiple of the initial volume. *Callus colour* was determined as green, white, yellow and brown. Brown calli were classified as not living whereas the two categories yellow and white were still living, even though they did not produce chlorophyll. The measurement of callus colour is the relative frequency of green calli among all calli of the same genotype and grown in the same environment.

In the graphs presenting the reaction norms (genotypic response functions, Figs. 1a and 2a) and the environmental response functions (Figs. 1b and 2b) the following symbols are applied: crosses (×) represent G7, triangles (▲) mark G8 and dots (●) stand for G9. The graph of the reaction norm is organized in such a way that the responses of one genotype to the different environments are chosen as a reference and plotted on the abscissa. In doing so, a linear ordering of the environments is specified and the norms of the other genotypes are plotted against this reference. The graphs for the environmental response functions are organized in an analogous fashion; here one of the environmental conditions was chosen to achieve a linear ordering of the genotypes on the abscissa, and the response functions of the remaining environments are plotted against this reference. The advantages of this method of representation are discussed in Gregorius (1977) and Gregorius and Namkoong (1985).

Results

Callus growth

a) Genotypic response functions (norms of reaction). Figure 1 a shows the reaction norms of the three genotypes with regard to the character callus growth and the environmental factor medium. Each measurement reflects the average performance of a genotype in its environment. The reference genotype is G9. Recall that media M1, M3, M5 contain sucrose without additional starch, whereas media M2, M4, M6 contain glucose with starch.

None of the effects of the genotypes is consistent over the whole range of environments. However, when restricted to the set (M3, M5, M6) of environmental conditions all genotypic effects are consistent, and thus separability of the genotypic effects is realized. In particular, the effects of G8 and G9 are statistically identical, while the effect of G7 ranks statistically significantly over the effects of G8 and G9. In the complementary set (M1, M2, M4), separability of genotypic effects is also realized with G7 and G9 being statistically identical and G8 being statistically significantly below the first two genotypic effects. All these results are based on pairwise comparisons with the help of the "Student"-test (Sachs 1974).

On the other hand, the reaction norm of G7 is consistently above that of G8 for all environmental conditions (according to "Student"-test). Hence, the norm of G9 partially resembles that of G7 and G8, but on complementary sets of environments. There seems to be also a rough tendency for G7 to be superior to G9, and G9 to be superior to G8 in growth.

b) Environmental response functions. Figure 1 b gives the environmental response functions where M2 is chosen as the reference environment.

It is seen that each response function is crossed by at least one other such function, so that none of the environmental effects is consistently distinguishable from any other. In several extreme cases it happens that the response function of one environment crosses four of the remaining five functions. Hence, we may conclude that the environmental effects show pronounced interaction with the genotypic effects in the sense that none of the media has an effect on callus growth which is for all genotypes consistently different from the effects of the other media.

Callus colour

a) Genotypic response functions (norms of reaction). Recall that colour is measured in terms of the relative frequency of green calli among all calli grown in the same environment (medium type) and having the same genotype. The reaction norms of the three genotypes are presented in Fig. 2 a, and G8 is the reference genotype.

The reaction norm of G7 ranks clearly above that of G9, and thus the effects of the two genotypes are properly distinct. Moreover, it is remarkable that the two reaction norms are almost completely parallel, so that the genetic difference between G7 and G9 appears

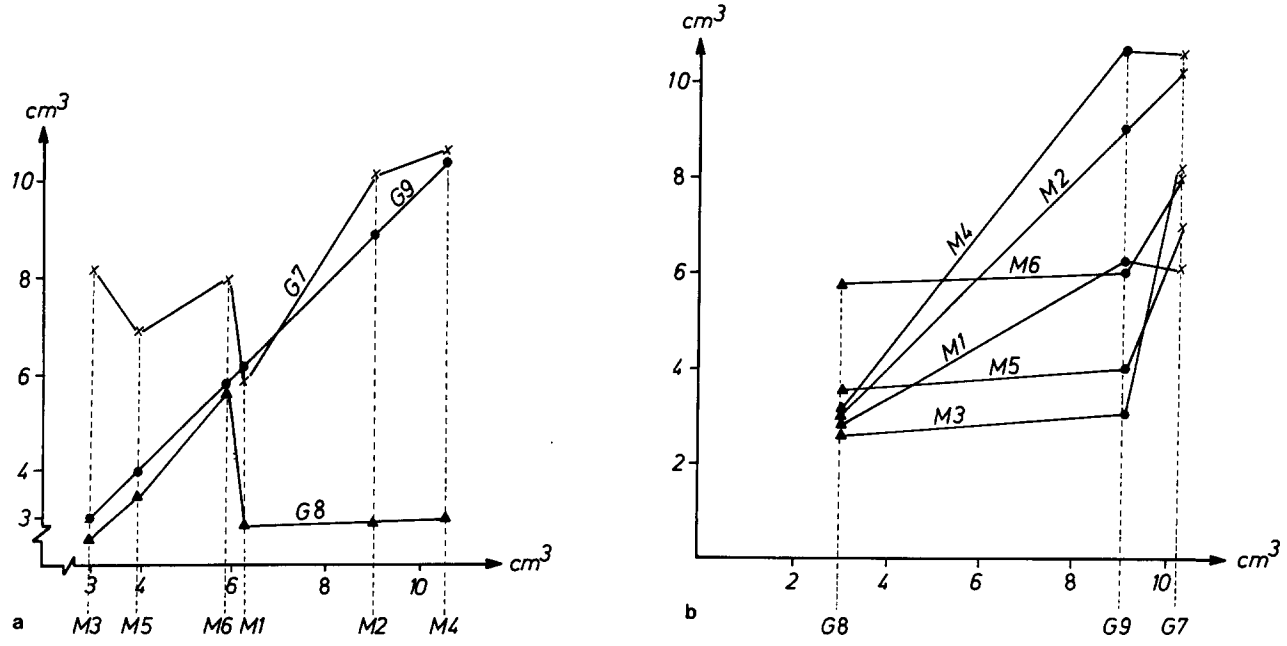


Fig. 1. **a** Norms of reaction for callus growth; **b** Environmental response functions for callus growth

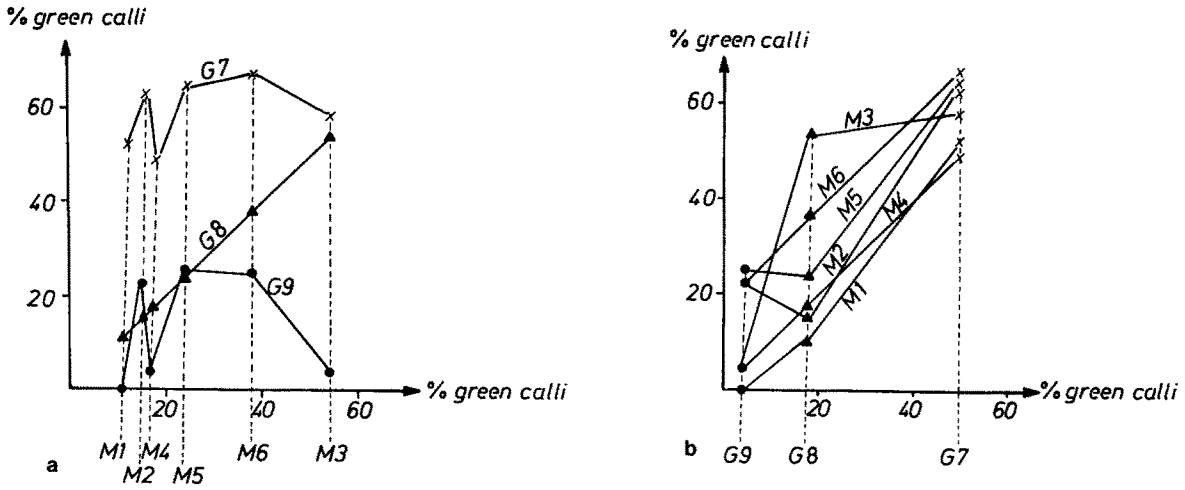


Fig. 2. **a** Norms of reaction for percentage green calli; **b** Environmental response functions for percentage green calli

to result in a constant shift in response to all six environments.

Since the norms of G8 and G9 show several crossings as well as distinct differences, their effects cannot be classified as consistently different over the whole range of environments. On the other hand, the effect of G8 is almost consistently different from that of G7 with the exception of M3, where the difference is statistically not significant (according to the χ^2-test). Thus, the genotypic effects are not separable as a whole, but some genotypes are clearly distinct from others in their effects. Yet there appears to be a tendency for G7 to produce the most, for G8 to produce the second most, and for G9 to produce the fewest green calli.

b) Environmental response function. The response functions are given in Fig. 2b with M4 as the reference environment.

At first glance, the picture appears much the same as in Fig. 1b insofar as crossings of functions are concerned. However, closer inspection reveals that the effect of M6 is consistently different from M1 and M4, where the effects of the latter two are statistically identical. Yet, as a whole, there is again a clear interaction of the environmental with the genotypic effects, although the interaction does not seem to be as strong as it is for growth.

Discussion

What is probably the most interesting result is that for both characters, the genotypes appear to be more consistent in their effects over the whole range of the six environments than are the environments in their effects over only half that number of genotypes. In other words, for the present experiment it appears that genetic differences between calli are less likely to be masked by the environment in their effects on the phenotypes; it is more likely that environmental differences are masked by the genotype. This is what is commonly understood by genotpe-environment interaction, and in our experiment it turns out that it is biased to one side in that the genotypes interact only weakly with the environment but the environment interacts strongly with the genotypes. As far as we know, the description of such one-sided interactions is entirely new, and not only in the field of tissue culture. However, in tissue culture they are of particular importance since most work in this field tends to ignore genetic variation within species (for exceptions see Ahuja 1983; Reddy et al. 1985; Pan et al. 1985) and concentrate almost exclusively on the effects of different media, light conditions, etc., on among others, environmental conditions. The present results clearly demonstrate that cases may exist in which the behaviour of a culture is determined to a larger degree by the selection of genotypes than by the selection of environmental conditions. This follows from the fact that under the present conditions and for both characters, callus growth and callus colour, the genotypic effects show a greater tendency to be consistent than the environmental effects, or in other words, genotypes interact to a lesser degree with the environments than do the environments with the genotypes.

Having established the strongly discriminatory effects of the genotypes, it is now appropriate to relate the genetic differences between calli (as they are suggested by the different degrees of kinship) to their performance with respect to growth and colour. For this purpose recall that G7 and G8 are unrelated, G7 and G9 are half sibs with a common pollen parent, and

G8 and G9 are 'cousins', the seed parents of which are full sibs. Hence, considering nuclear genes, G7 and G9 are expected to be genetically more similar than G8 and G9. On the other hand, considering extranuclear genes which are transmitted via the ovules only (birch is not known to be a species with biparental transmission of extranuclear genetic information), G8 and G9 are even likely to be genetically identical, since their mothers are full sibs. For the other two pairs such extranuclear relationships are not known. This information has to be accounted for when trying to explain the different relationships of the reaction norms in callus growth and in callus colour (compare Fig. 1 a and 2 a). Growth is generally believed to be under predominantly nuclear control, and colour is likely to be affected by the extranuclear genes present in the chloroplasts. (Colijn et al. 1983).

These expectations are reflected with surprising clarity in our data. For callus growth the norms of the unrelated genotypes G7 and G8 are clearly separated, while the norm of G9 resembles that of G8 in some environments and that of G7 in others. This is in complete accordance with the fact that G9 is expected to show some genetic similarity with both G7 and G8, while G7 and G9 are not expected to be genetically similar.

With respect to callus colour the situation is a bit more intricate. Yet, the expected extranuclear genetic identity of G8 and G9 may be considered to be responsible for the partial resemblance of their norms. The observation that the norms of G7 and G9 are clearly distinct and that the same holds for G7 and G8 (however to a lesser degree) can also be explained by the fact that each of these two pairs is expected to differ in their extranuclear genes. The difficulty lies in explaining why G7 and G9 are located so far apart and are almost parallel in their norms. A tentative interpretation may be deduced by considering that G7 and G9 show the strongest nuclear relationship but may differ substantially in their extranuclear genes. The nuclear similarity might explain the similarity in shape of the norms, and the extranuclear difference might cause the shift. However, substantiation of these suggestions would require the consideration of larger numbers of genotypes.

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