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APPLICATION OF INTUITIONISTIC FUZZY SET IN PLANT TISSUE CULTURE AND *IN VITRO* SELECTION

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INTRODUCTION

The process of plant regeneration includes three stages: (1) **callusogenesis**, the formation and growth of undifferentiated plant tissue (callus), (2) **embryogenesis**, initiation of differentiated structures (embryos) from callus and (3) formation of viable plantlets from embryos. Experimental parameters influencing this process are: plant genotype, initial explant (type, developmental stage, and physiological state), components in the nutrient medium, conditions of cultivation (temperature, humidity, and light intensity), and duration of individual stages [2]. *In vitro* plant regeneration aims at obtaining maximal number of regenerated plants by introducing such changes in the above parameters that optimize the process for the specific plant species, cultivar, or genotype at hand.

In the field of *in vitro* selection of plant tissue cultures for resistance to diseases, selective agents such as purified toxin or culture filtrate containing toxic substances, released during cultivation of the pathogen, are used. Selection is exerted by including the selective agent in the nutrient media used for *in vitro* cultivation. Efficient selection requires maximal realization of two processes with opposite effects: (a) employment of strong selective agents (high concentrations of toxins or culture filtrates) that cause high tissue mortality and (b) regeneration of high number of plants that requires viable tissue obtained after selection. Calli derived from different plant cultivars behave differently with respect to concentrations of selective agent and subsequent regenerative ability. In order to optimize the selection process, the concentration of selective agent must be adjusted for each cultivar so that a strong selection is achieved but there remains enough viable callus tissue to regenerate sufficient number of plants to be used for further screening for resistance in the field [14].

MODELS FOR TISSUE CULTURE AND *IN VITRO* SELECTION

Here we propose an approach for optimizing plant regeneration and *in vitro* selection that is based on the apparatus of Intuitionistic Fuzzy Set (IFS) and Intuitionistic Fuzzy Logic (IFL) [6,8]. We will develop two models: (1) for the process of plant regeneration and (2) for the *in vitro* selection.

The first model is based on data obtained using different combinations of experimental parameters [13]. Each of these parameters has a set of values that can be defined and limited on the basis of previous data. We will assume that each observation is made on a system with a specific combination of values for different parameters. The number of observations can be decreased by choosing the variable parameters on the basis of their relative importance and/or excluding those parameters that have already been optimized. This implies that the experimental parameters can be changed in a controlled fashion at the outset and during the experiment as is usually the case. The efficiency of the regeneration process can be judged on the basis of several criteria such as callus weight, callus viability, callus color, callus texture, number of embryogenic structures, form of embryos, growth rate of embryos, number of regenerated plants, viability of regenerated plants *etc*. The uncertainty in determining the optimal experimental parameter decreases with increasing the number of criteria evaluated.

Many of the above criteria are evaluated by measurable quantities, representing a gradual change from undesirable to desirable responses, or *vice versa*. In these cases, we can assign "intuitionistic" upper and lower limits, a and b, such that if a measurable quantity, q, falls between these limits, that is, $b \le q \le a$, then we cannot say unambiguously whether it is a "good" or "poor" response. Conversely, values outside the "intuitionistic" limits can be unambiguously assigned to one of the two categories.

Let criteria C_1, C_2, ..., C_k be given and let the observations O_1, O_2, ..., O_n, be made according to the above criteria on systems with different combinations of parametric values. We can assemble the following index matrix (IM) [4]

$$(1)\qquad \begin{array}{c|ccc} & C_1 & \cdots & C_k \\ \hline O_1 & \langle \alpha_{1,1}, \beta_{1,1} \rangle & \cdots & \langle \alpha_{k,1}, \beta_{k,1} \rangle \\ \vdots & \vdots & \ddots & \vdots \\ O_n & \langle \alpha_{1,n}, \beta_{1,n} \rangle & \cdots & \langle \alpha_{k,n}, \beta_{k,n} \rangle \end{array}$$

where $\alpha_{i,j}$ and $\beta_{i,j}$ are the parts of the unambiguous "good" and "poor" responses, respectively, that is, $\alpha_{i,j} = a_{i,j}/N$ and $\beta_{i,j} = b_{i,j}/N$ where $a_{i,j}$ and $b_{i,j}$ are the numbers of "good" and "poor" calli, embryos, or plants and N is the total number observed. It follows from the definition that $\alpha_{i,j} + \beta_{i,j} \le 1$ and $\alpha_{i,j}$, $\beta_{i,j} \in [0,1]$ for every $1 \le i \le k$ and $1 \le j \le n$. Practically, we construct an IM which represents the relation between the universes $\{O_1, O_2, ..., O_n\}$ and $\{C_1, C_2, ..., C_k\}$ in the sense of [3,9-11].

We can then define the following intuitionistic fuzzy group of values:

$$(2)\qquad \begin{aligned} \overline{O}_{1,j} &= \left\langle \max_i(\alpha_{i,j}), \min_i(\beta_{i,j}) \right\rangle \\ \overline{O}_{2,j} &= \left\langle \frac{\sum_i \alpha_{i,j}}{k}, \frac{\sum_i \beta_{i,j}}{k} \right\rangle \qquad (1 \le j \le n) \\ \overline{O}_{3,j} &= \left\langle \min_i(\alpha_{i,j}), \max_i(\beta_{i,j}) \right\rangle \end{aligned}$$

which correspond, respectively, to the optimistic, averaged, and pessimistic prognoses for the outcome of regeneration about the j-th criterion (cf. [1,5]). The above values allow us to compare responses from the whole set of observations to a given criterion. Criteria for regeneration can be rated in order of importance, the most important being, for instance, number of regenerated plants. Then, sets of observations can be evaluated according to the number of regenerants obtained for each set, then according to the next most important criterion, *e.g.* plant viability, and so on.

On the other hand, we can build the following fuzzy group of values:

$$\overline{C}_{1,i} = \left\langle \max_j(\alpha_{i,j}), \min_j(\beta_{i,j}) \right\rangle$$

$$(3) \qquad \overline{C}_{2,i} = \left\langle \frac{\sum_j \alpha_{i,j}}{n}, \frac{\sum_j \beta_{i,j}}{n} \right\rangle \qquad (1 \leq i \leq k)$$

$$\overline{C}_{3,i} = \left\langle \min_j(\alpha_{i,j}), \max_j(\beta_{i,j}) \right\rangle$$

As a result we can make inferences about the best and worst combinations of parametric values across all criteria and the worst combinations will be excluded from further experiments. More consideration will be given to the observations with optimal combinations of values and those will be further specified by dividing the respective parameters into smaller increments.

On the basis of the above values (3), we can determine which observation satisfies our criteria to the greatest degree with the use of the relationship:

$$(4) \qquad \langle a, b\rangle \geq \langle c, d\rangle \text{ if } a \geq c \text{ and } b \leq d$$

If a maximal element with the above property cannot be defined, then we can use the norm σ_3 (see [15]):

$$(5) \qquad \sigma_3(a,b) = \frac{a+1-b}{2}$$

that will enable us to identify the observation with the optimal combination of parametric values to achieve the best regeneration.

For each $\alpha_{i,j}$ and $\beta_{i,j}$ we can define the ambiguity, $\pi_{i,j}$: $\pi_{i,j} = 1 - \alpha_{i,j} - \beta_{i,j}$, which represents the part of ambiguous calli, embryos, or plants. Following [5] we can construct the intuitionistic fuzzy groups (for the operator **F** see [7]):

$$(6) \qquad \overline{\overline{O}}_{1,j} = \mathbf{F}_{\alpha_{1,j},\beta_{1,j}}\left(\ldots\left(\mathbf{F}_{\alpha_{s-1,j},\beta_{s-1,j}}\left(\mathbf{F}_{\alpha_{s+1,j},\beta_{s+1,j}}\left(\ldots\left(\mathbf{F}_{\alpha_{k,j},\beta_{k,j}}\left(\left\langle \alpha_{s,j}, \beta_{s,j}\right\rangle\right)\right)\ldots\right), \quad (1 \leq j \leq n),$$

where the couple $\left\langle \alpha_{s,j}, \beta_{s,j}\right\rangle$ corresponds to the s-th criterion C_s for which the j-th observation O_j has the greatest ambiguity. The **F** operator decreases the ambiguity of the elements to which it is applied. Thus, the fuzzy groups $\overline{\overline{O}}_{1,j}$ enable us to make less ambiguous evaluations for selected criteria and observations.

The second model concerns *in vitro* selection. This process can be divided in 1) callusogenesis, 2) selection, 3) embryogenesis, and 4) formation of regenerated plants [12]. As we can see, this is essentially a process of regeneration that includes a stage of selection after callusogenesis. Therefore, the reasoning outlined in the first model applies also here. We should take into account, however, the additional stage. First, we include additional criteria or modify some of the regeneration criteria. For instance, the meaning of the criteria pertaining to the callus tissue, such as callus weight and callus color can be reversed so that low callus weight and high number of discoloured calli favour selection. Second, we should include the concentration of selective agent as one of the most important parameters. Third, we must also have in mind that efficient selection, estimated by some of the criteria for stage 2) leads to poor responses to other criteria pertaining to stages 3) and 4) of regeneration. Therefore, we must take into account all the values in the fuzzy groups (3) and (4), whereas in the first model it is usually sufficient to consider only the values for the optimistic prognosis.

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