Advanced approaches of cell selection and new forms of cell lines with combined stress tolerance

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Cell selection is a reliable method for obtaining plant forms with genetic changes. Global environment deterioration and enlarged deficit of stress tolerant genotypes call for new ideas and approaches. The idea of using heavy metal ions in cell selection for obtaining variants tolerant to abiotic stresses was created. Heavy metal ions (HMI), a large number of toxicants, are divided into two categories. The first group joints physiological ions. The second one consists of ions, toxic at trace concentrations. They are: Pb^{2+}, Ba^{2+}, Cd^{2+}, etc. it is known that barium (Ba^{2+}) ions disturb cell K^+ transport. At the same time the disturbance of K^+/Na^+ ratio is the pathologic result of the salinity. Cadmium (Cd^{2+}) ions inflict plant water status. So we used those cations for obtaining osmotic stress tolerant variants via cell selection. Selective systems with lethal for wild type cell cultures doses of Ba^{2+}or Cd^{2+} cations were elaborated. Suspension cell cultures were plated between two layers of selective media in Petri dishes. After 30-35 days of cultivation the growth of single cells started. The appearance frequency was 10^{-6}. Ion-resistant cell proliferated and formed certain resistant cell lines. After several (2-4) passages under initial stress conditions calli biomass were divided and transferred to fresh media of different compositions. They are: standard nutritional media (normal conditions); media with the addition of HMI (stress I, mineral); media with the addition of sea water salts or mannitol (stress II, osmotic). All stress-formed ingredients were added at lethal for ordinary cultures doses. Several resistant cell lines of tobacco, soybean, wheat, maize were selected. Those lines challenged any abiotic stress. The media rotations were arbitrary. Cell selection with HMI is the advanced method for obtaining plants with combined tolerance to abiotic stresses.

Effect of low-temperature exposure on the morphogenesis reactions of the callus culture of winter wheat

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The culture of plant cells and tissues plays a significant role in studying the morphogenesis pathways, since the widest range of plant morphogenetic potential appears *in vitro.* A complex of endogenous and exogenous factors determines the program of morphogenesis, among which temperature is one of the most important. For winter wheat, the period of prolonged exposure of low positive temperature (vernalization) is required for transition to generative development, i.e. full realization of morphogenesis. The effect of low-temperature exposure on callus culture of winter wheat, perhaps, will also stimulate the pathways of morphogenesis *in vitro.* The aim of the study was to investigate the effect of positive low-temperature exposure (15 and 30 days) on the morphogenetic reactions of callus culture of three winter wheat varieties - Doridna, Statna and Astet. After low-temperature exposure, the calli were transferred to the regeneration medium MC, supplemented with 3 mg/l BAP and 0,5 mg/l NAA. Calli had been being cultured on the light intensity of 2-3 kL for 4 weeks. According to the results, all investigated variants of winter wheat varieties exhibited morphogenetic reactions, however in varying degrees.

Callus of Doridna was light brown, friable, watered, with green roots; Statna and Astet - matte, white and yellow, with green chlorophyllous areas and roots, friable and watered. Organogenesis was manifested by the formation of meristematic zones and intensive rhizogenesis. While the formation of roots was more intense than gemogenesis in all varieties irrespective of temperature conditions. Chlorophyllogenesis was manifested in different ways: the formation of green meristematic zones, green roots (abnormal path of development) and general greening of callus tissue (formation of mixotrophic callus). In all experiment variants subjected to + 4° C temperature for both 15 and 30 days, the increase of frequency of meristematic zones formation was observed. However, the increase of number of those zones per callus was found only in Statna. The maximum intensity of gemogenesis was shown for Astet, the minimum - Doridna. In general, low-temperature exposure, regardless of duration, stimulated morphogenetic reactions of calli of the winter wheat varieties.

Продуктивность водоросли *Haematococcus pluvialis*, содержание в ней фотосинтетических пигментов, активных форм кислорода и астаксантина при выращивании в условиях засоления

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Штамм IBCE H-17 водоросли *Haematococcus pluvialis* из коллекции Института биофизики и клеточной инженерии НАН Беларуси был изучен на предмет индукции накопления в клетках кето-каротиноида астаксантина в условиях избыточного засоления питательной среды 25, 50, 100 200 и 300 мМ NaCl. Параллельно оценивали продуктивность *H. pluvialis* по показателям сухой биомассы и белка, содержания фотосинтетических пигментов и АФК. NaCl в концентрациях 25, 50 и 100 мМ стимулировал накопление сухой биомассы водоросли в течение 12-и суток выращивания в среднем в 1,3 раза по сравнению с контролем. Содержание белка в расчете на сухую биомассу снижалось и составляло в среднем 70% от контроля на 7-е сутки культивирования при использовании 50–300 мМ соли и 55% – на 12-е сутки для концентраций соли 100–300 мМ. Через 7 суток выращивания на растворах NaCl уменьшалось и общее количество фотосинтетических пигментов – хлорофилла *a* и *b*, а также каротиноидов – неоксантина, виолаксантина, лютеина и β-каротина. Хлорофилл *b* оказался более устойчивым к засолению по сравнению с хлорофиллом *a*. Наиболее сильно под воздействием NaCl снижался уровень β-каротина. Стрессовые условия, создаваемые NaCl, привели к генерации АФК. Так, через 7 суток культивирования общее содержание АФК в варианте «NaCl-100» в 1,7 раза превышало таковое в контроле и в 3,0 раза выше контроля в 12-суточной культуре. Отмечено существенное положительное влияние NaCl на содержание астаксантина. Максимальный эффект наблюдали при использовании 100 мМ NaCl. Через 7 суток культивирования содержание астаксантина превышало контрольные показатели в 2,8 раза, а через 12 – в 20,5 раз. Количество клеток водоросли через 7 суток выращивания в варианте «NaCl-100» уменьшалось в среднем на 33%, в то время как диаметр клеток возрастал на 29%.