Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

JEZS

Journal of Entomology and Zoology Studies

e-ISSN: 2320-7078
p-ISSN: 2349-6800
JEZS 2014; 2(3): 236-239

Received: 13-04-2014
Accepted: 29-05-2014

S. Yerzhanova
PhD, Assoc. Professor – leading researcher of the Department of fodder and oil crops, "Kazakh Research Institute of Agriculture and Plant Growing, sakyshyer@mail.ru, 8727-3883925, 8 7752249782.

G. Meyrman
Dr., Professor, Academic of National Academy in Republic of Kazakhstan – Head of the Department of fodder and oil crops, "Kazakh Research Institute of Agriculture and Plant Growing", meirman07@rambler.ru, 8727-3883925.

A. Abugalieva
Dr., Professor - Head of the Laboratory of Biochemistry and quality of plants, "Kazakh Research Institute of Agriculture and Plant Growing, abugalieva@mail.ru, 8727 -3883925.

B. Bashabaeva
PhD – Senior researcher of the Laboratory of Biochemistry and quality of plants, "Kazakh Research Institute of Agriculture and Plant Growing", bashabaeva@mail.ru, 8727-3883925.

A. Abekova
PhD - Senior researcher of the Laboratory of Biotechnology of Plant, "Kazakh Research Institute of Agriculture and Plant Growing, aabekova@ mail.ru, 8727-3883925.

R. Massonichich - Shotunova
PhD - Senior researcher of the Department of fodder and oil crops, "Kazakh Research Institute of Agriculture and Plant", rausana2010@mail.ru, 8727-3883925.

S. Abayev
PhD - Senior researcher of the Department of fodder and oil crops, "Kazakh Research Institute of Agriculture and Plant", serik_abayev@mail.ru, 8727-3883925.

Correspondence:
S. Yerzhanova
Department of fodder and oil crops, "Kazakh Research Institute of Agriculture and Plant Growing", 040909, Republic of Kazakhstan, Almaty region, Karasay district, Almalybak, Erlepesov st. 1

Possibility to Micropropagation Vetch *(Vicia Sp.)*

***S. Yerzhanova, G. Meyrman, A. Abugalieva, B. Bashabaeva, A. Abekova, R. Massonichich - Shotunova, S. Abayev**

ABSTRACT
Vetch *(Vicia sp.)* One of the most important crop of the world. In Kazakhstan is a traditional and major source of legume fodders. In this work, we studied the regeneration ability of genotypes of vetch explants on media with the different concentrations of cytokinin and auxin for activation of axillary buds and obtaining regenerants. Optimized 12 culture media to culture Vetch *(Vicia sp.) in vitro* genotypes. The frequency of shoot regeneration, depending on concentration of cytokinin and auxin, was 1.0 - 10.8%. On these explants were observed multiple shoot formation.

Keywords: Vetch (*Vicia sp.*), *in vitro*, micropropagation, mature and immature embryos, regeneration of plants.

1. Introduction
The vetch (*Vicia Sp.*) is one of the major annual legume fodders. Green mass vika contains up 18.6% of the protein, in the seeds to 37% or more [1]. The protein concentration in the grain and straw of some varieties is higher than 300 and 100 g/ kg, respectively [2, 3]. It has a high content of water-soluble fraction, so that the food of the vetch *(Vicia Sp.)* (Green forage, hay, haulage, silage) are easily digested when eaten by the animals and is essential to strengthen the food supply by increasing the protein content of the feed. The prospect of this culture is marked by foreign authors [4, 5]. Scientists from Spain are conducting research on mechanized harvesting [2], ICARDA scientists are conducting research fodder value vetch *(Vicia Sp.)* for livestock in arid regions such as Central and West Asia, North Africa and South Australia [6, 7] and vetch has a good adaptability and productivity in high mountains of China and Mexico [8, 9].
According to the literature All - Russian Research Institute of leguminous plants developed cloning technology in pea *Pisum sativum L.*, broad beans *Vicia faba L.*, buckwheat *Fagopyrum esculentum Moench.*, Millet *Panicum miliaceum L.,* Payziev *Echinochloa frumentacea L.*, as well as some rare species of leguminous and cereal crops [10].
There is a shortage in Kazakhstan seeds vetch (Vicia Sp.). In this connection one way to quickly reproduction individual genotypes of vetch (Vicia Sp.) Is a clonal micropropagation (apical meristem, shoots and immature embryos).
An integrated approasch to the study of genetic diversity in the vetch *(Vicia Sp.)* with the traditional method of selection and one of the most promising technique with biotechnological method is robust and will allow for the transfer of breeding culture to a new level.
Technology clonal propagation *in vitro* was developed in the world for more than 2 400 species of plants [11, 12, 13].
Inbreeding practices are increasingly being used for genetic improvement of plants, based on the methods of *in vitro* [14, 15, 16, 17, 18]. However, the technology must be continuously improved. Their main drawbacks includes: requirement of large labor and high cost of energy, materials and other resources, the need for automation of all processes.
Development of biotechnological method for rapid multiplication of valuable genotypes vetch *(Vicia Sp.)* is relevant, because of its economic importance and the extended growing season.

2. Materials and Methods
The plants were grown in a greenhouse conditions at 5000 lux illumination, the light for 16 h and a temperature of 24 °C. For the preparation of culture media and sterilization of material, too ls were used by the general methodological principles and techniques [19].

The correct choice of explants with the growing conditions and phase of development of the plant i.e. - the donor is important. Explants and embryos from donor of Vetch plants were collected for further processing.

Sterilization of vetch beans was carried with 96% ethanol, 5 minutes, followed by rinsing three times in sterile distilled water for 3 minutes. Under sterile conditions, insulation wire immature and mature embryos (*Vicia Sp*.) Landing on a nutrient medium.

Nutritive medium for the culture of plant tissues have a complex composition, which is optimized to obtain a successful induction of plant regeneration. The main ingredients are mineral salts (macro-and microelements, a carbohydrate source (sucrose, etc.), vitamins, amino acids, various growth factors, agar. For culturing embryos and explants vetch the artificial medium Murashige-Skoog (MS) medium is used that is modified by the addition of various biologically active substances (20). After four weeks, the plants were formed and these regenerants were transferred to rooting medium in sterile plastic or glass boxes. Statistical analysis was performed using the software package STAT (KIZ, 1986), [21].

3. Results and Discussion

Explants mature and immature embryos were placed on MS medium supplemented with different concentrations of cytokinin activation of axillary buds. Best proliferation of axillary meristems observed on the medium with 6-benzylaminopurine (BAP), (table 1). Multiplication increased in passage 4 in a medium with 0.5 BAP 3.0 ml/l. In the presence of this concentration was observed bookmark microshoots they had to be passaged onto fresh medium. With increasing BAP in the medium to 3.0 ml/l decreased the formation of additional kidney was observed vitrification shoots. The induction of shoot celebrated a 6-8 day. After 23-25 days of receiving the fully-formed plant.

Table 1: Effect of cytokinins on the activation of axillary meristems (*Vicia Sp*.) in *in vitro*

The concentration of plant hormones, ml/l	Colouring escape	Growth of the main shoot length, mm	The multiplication factor
BAP 0.5	Green	5.0 ±1.38	0.6
BAP 1.0	Green	10.8 ±1.52	1.23
BAP 2.0	Green	7.1 ±1.43	1.12
BAP 3.0	Green	3.0 ±1.12	0.4
Kinetin 0.5	Dark - green	4.0 ±1.15	0.8
Kinetin 1.0	Dark – green	8.0 ±1.24	1.16
Kinetin 1.5	Dark - green	2.0 ±1.04	0.4
Without hormone	Green	3.0 ±1.11	0.6

Then shoots were transplanted to MS medium (without hormones) for their development. Microshoots developed normally.

To initiate the development of the roots, substances such as auxin action: indoline-acetic acid (IAA), naphthyl acetic acid (NAA) and indole butyric acid (IBA) were used. 12 types of media were tested (table 2) at the rooting stage, where the main medium containing 2% sucrose, ½ of makrosoli MS.

Table 2: Effect of IBA, NAA and IAA on rooting vetch under *in vitro* conditions

Option	Auxin concentration, ml/l	Characteristics of cultures	Rooting, %	the number of roots per shoot, pcs	The average length of the root system, mm	Callus formation of shoots
1	IBA 0.5	Intensive elongation of roots	40	2	35	+
2	IBA 1.0	Intensive elongation of roots	65	4	90	+
3	IBA 3.0	Intensive elongation of roots	15.5	1	13	+
4	NAA 0.5		37	3	4	-
5	NAA 1.0		60	8	70	-
6	NAA 3.0		12	1	4	-
7	IAA 0.5	Elongation of shoots	-	2	10	
8	IAA 1.0	Elongation of shoots	-	6	20	
9	IAA 3.0	Elongation of shoots	-	1	5	
10	IBA + NAA 2.0+2.0		50.0	5	130	⊥
11	IBA+NAA 2.0+1.0		35.0	3	60	
12	IBA+NAA 1.0+1.0		27.0	1	10	

Positive results were obtained rooting the vetch on variants containing NAA 1.0, IBA - 1.0, IBA + NAA – 2.0 +2.0 ml/l (Figure 1). All test concentrations of IBA caused rapid growth of shoots in the Vicia Sp. (Figure 2). The maximum elongation of shoots was observed at 1.0 ml/l IBA.

Fig 1: Regenerated plants in medium with NAA 1.0 ml/l

Fig 2: Regenerated plants 0.5ml/l IAA

Survival of isolated nuclei Vicia Sp. depends on the age of embryos, composition of the nutrient medium. The developed method of micropropagation Vicia Sp. can be used to quickly produce valuable endangered species and hybrids.

Fig 3: Callusogenesis (Callus-formation) vetch *(Vicia Sp*.)

All rooms vetch basically gave direct regeneration, with the exception of the second option, in which were found calluses (induction of callus formation is equal to 3.43 ± 0.01 (Figure 3). Obtained calluses were transplanted to the environment for plant regeneration. Along with green plants is regenerated albino without chlorophyll regenerates.

For the best adaptation of plants to the environment in 5-7 days covered with glass caps, and at night opened (Figure 4).

Fig 4: Plants - regenerates vetch *(Vicia Sp*.)

4. Conclusion

Value is determined what Vicia Sp. can produce a high yield of green mass (400-500 kg/ha) and seed (30-40 kg/ha), readily eaten by all sorts of farm animals. According to the content of protein in the green mass (3-3.5%) Vicia Sp. is superior to all other legumes, but by its content in seeds (25-30%) is second only to lupine.

Was selected one of the major steps in implementing this technology Vicia Sp. Was selected sterilizing agents for explants and embryos (Sterilization of Vicia Sp. beans was carried with 96% ethanol, 5 minutes followed by rinsing three times in sterile distilled water for 3 minutes), the optimal concentration of cytokinin to increase the multiplication factor axillary shoots (positive results were obtained rooting the

vetch on variants containing NAA 1.0, IBA - 1.0 ml/l, IBA + NAA – 2.0 +2.0 ml/l.). Identified favorable ratio of growth promoters (The maximum elongation of shoots was observed at 1.0 ml/l IBA.).

5. Acknowledgement

This research was supported by the 212 programm, which is funded by the budget of the programm of the Ministry of Agriculture and the Ministry of Education and Science of the Republic of Kazakhstan. The authors are grateful to Academician of the Academy of Agricultural Sciences of the Republic of Kazakhstan S. B. Kenenbaev and Head of the Laboratory of Biotechnology Urazaliev K.R.

6. References

1. Kukresh LV. The formation of high yields of grain and green mass of spring wiki. Gorki, 1990, 246.
2. Determination of glutamyl-S-ethenyl-cysteine in narbon vetch (*Vicia narbonensis L.*) seeds by high performance liquid chromatography. http:/ www.elsevier.com. 2011.
3. Yerzhanova S, Meyrman G, Abugaliyeva A. Formation, the study of genetic resources and quality of forage crops in Kazakhstan. III Vavilov international conference "Ideas N. Vavilov in the modern world, "Dedicated. The 125th anniversary of N. Vavilov. 6-9 November, 2012, 155.
4. Jones MJ, Singh M. Long-term yield patterns in barley-based cropping systems in Northern Syria 2. The role of feed legumes. J Agric Sci Cambridge 2000; 135:237–249.
5. Saxena MC, Abd El, Moneim AM, Ratinam M. Vetches (Vicia spp.) and chicklings (Lathyrus spp.) in the farming systems in West Asia and North Africa and improvement of these crops at ICARDA. In: Garlinge, J.R., Perry, M.W. (Eds.), Potential for Vicia and Lathyrus Species as New Grain and Fodder Legumes for Southern Australia. Co-operative Research Centre for Legumes in Mediterranean Agriculture. Occasional Publication 1993; 1:2–9.
6. ICARDA. Narbon vetch: A Feed Legume for Low-Rainfall Areas. ICARDA Annual Report, Aleppo, Syria, 2006, 59–60.
7. Siddique KHM, Loss SP, Regan KL, Jettner RL. Adaptation and seed yield of cool season grain legumes in Mediterranean environments of south-western Australia. Aust J gric Res 1999; 50:375–387.
8. Nan ZB, Abd El, Moneim AM, Larbi A, Nie B. Productivity of vetches *(Vicia sp.)* Under alpine grassland conditions in China. Trop Grasslands 2006; 40:177-182.
9. Flores MA, Sanchez RA. Forage production of narbon bean entries under rainfed conditions. In: VI Simposio Internacional de Pastizales, 4-7 Noviembre, 2009.
10. Suvorova GN, Sobolev GV. The development and use of biotechnology techniques to create new forms of plant leguminous and cereal crops. SPJ "Legumes and cereal crops 2012; 2:10-13.
11. Muromtsev *et al*. Other basics of agricultural biotechnology. 1991, 350.
12. Bajaj YPS. Biotechnology of medicinal plants. In the Sun. Theses Internat. Conf. "Biology of plant cells *in vitro*, BT and the preservation of the gene pool, 1997, C.5.
13. Baiburina RK *et al*. The use of *in vitro* methods for propagation / V Sun. Theses 7 Internet. Conf. "Biology of plant cells *in vitro*, BT and the preservation of the gene pool, 1997, S.393.
14. Rakhimbaev I. Biotechnological methods in plant breeding. / In the Sun. "The biological basis of selection of crops." Almaty 1996, 18-34.
15. Abugaliev IA, Shegebaev OSh. Results and prospects of biotechnology research in crop KazNIIZ. /In the Sun. "Biotechnology in crop breeding." Almaty 1991, 3-16.
16. Yerzhanova ST. ST Alimgazinova Clonal micropropagation of Lavandula Spich H. / In the Sun. "Physiology and Biochemistry - integral link selection." Almaty 1998, 47-51.
17. Alimgazinova BSh, Yerzhanova ST. Clonal micropropagation lavender / Guidelines. Almaty 1999, 22.
18. Didorenko S, Absattarova A. Tissue culture of soybean *(Glycine Max. L.)* / Guidelines. Almalybak. 2008, 25.
19. Butenko RG. Culture isolated tissues and physiology of plant morphogenesis. Moscow: Nauka, 1964, 272.
20. Gambord OL, Murachige T, Thorpe TA, Vasil IK. Plant tissue culture media//*in vitro* 1976; 12:473-478.
21. The software package STAT/KIZ. Kazakhstan. Almalybak, 1986.