KARTOSHKA X VIRUSI ANTIGENI ASOSIDA TURLI IMMUNOLOGIK USULLAR VA IFA VARIANTLARI SEZGIRLIGINI ANIQLASH

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ANNOTATSIYA

Bugungi kungacha fitoviruslarni diagnostika qilishda bir qator usullar qo'llanilib kelingan bo'lib, ularning har biri o'ziga xos sezgirligi bilan bir-biridan farqlanadi. Bu usullar ichida immunologik usullar o'zining sezgirligi va bir vaqtning o'zida bir qancha namunani aniqlay olishi bilan afzalliklarga ega. So'ngi yillarda biotexnologiyaning rivojlanishi natijasida paydo bo'lgan immunologik usullar sezgirligining avvaldan qo'llanilib kelingan usullardan sezgirligi jihatdan yuqori darajada turishi haqidagi ma'lumotlar bir qator mualliflar tomonidan keltirib o'tilgan. Shuning uchun ushbu ishda tomchi, ikkiyoqlama immunodiffuziya, immunoferment analizi (IFA) usulining «to'g'ri», «noto'g'ri» va «sendvich» kabi variantlarining sezgirlik darajasi solishtirilgan hamda ularning ichidan IFAning sendvich variantining sezgirlik darajasi eng yuqori, ya'ni 10^{-9} ekanligi aniqlandi. Ushbu tajribalarda antigen sifatida kartoshka X-virusidan (KXV) foydalanildi.

Kalit so`zlar: Kartoshka X-virusi, immunoferment analizi, tomchi usuli, ikkiyoqlama immunodiffuziya, antigen, mozaika, antitana, antizardob.

DETERMINATION OF THE SENSITIVITY OF DIFFERENT IMMUNOLOGICAL METHODS AND ELISA VARIANTS BASED ON ANTIGEN POTATO VIRUS X

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ABSTRACT

To date, a number of methods are used to diagnose phytoviruses, each of which has its own specific sensitivity. Among these methods, immunological methods have

the advantage of their sensitivity and ability to simultaneously detect multiple samples. In recent years, a number of authors have reported that the sensitivity of immunological methods that have emerged as a result of the development of biotechnology is higher than that of previously used methods.

Thus, this study compared the sensitivity levels of drip, bilateral immunodiffusion and “correct”, “incorrect” and “sandwich” variants of the enzyme immunoassay (IFA) method, and the sandwich version of IFA had the highest sensitivity, ie 10^{-9}. Potato Virus X (PVX) was used as antigen in these experiments.

Keywords: Potato virus X, enzyme immunoassay, immunodiffusion, antigen, mosaic, antibodies.

KIRISH

Bugungi kungacha o‘simlik viruslarini diagnostika qilishda indikator o‘simliklar, kiritmalarga asoslangan, elektronmikroskopiya va immunologiya kabi bir qator usullar qo‘llanilib kelinmoqda, ularning ichida immunologiyaga asoslangan usullar o‘zining sezgirligi, tezkorligi va bir qator qulayliklari bilan alohida ajralib turadi, ammo oxirgi yillarda biotexnologiyaning, ayniqsa analitik biotexnologiyaning rivojlanishi natijasida immunologik usullarning sezgir va tezkor turlari yaratilmoqda. Amaliyotda bu usullarning qo’llashining o’ziga xos qiyinchiliklari va talab etiladigan metodologiyasi mavjud bo’lib, tadqiqotchidan bu usullarni chiqur bilish talab etiladi.

ADABIYOTLAR TAHLILI VA METODOLOGIYA

Immunologik usullar bir vaqtning o‘zida bir nechta namunani aniqlay olishi va tezkorligi, sezgirligi bilan boshqa usullardan ajralib turadi [2, 4, 8, 12]. Bunday usullar qatoriga IFA usulini kiritishimiz mumkin, usulning bugungi kungacha ishlab chiqilgan bir qator turlari mavjud bo‘lib, ularning ichida oxirgi yillarda viruslarni diagnostika qilishda geterogen turining bir nechta variantlari qo‘llanilib kelinmoqda [3, 6, 7, 9]. Bu variantlar va ba’zi immunologik usullar sezgirligini solishtirish hamda ularning ichidan eng sezgir va qulayini aniqlash muhim masalalardan biri hisoblanadi [1, 5, 8, 11, 14]. Buni aniqlash maqsadida suyultirish orqali tayyorlangan AG namunasi olinib IFA ning to‘g‘ri, noto‘g‘ri va sendvich variantlari hamda IID va TU yordamida tekshirilib ko‘rildi va bu usullarning sezgirligi solishtirildi. Tekshirishda AG sifatida KXV dan foydalanildi.

Ushbu ishda tomchi usuli, ikkiyoqlama immunodiffuziya, IFAning «to‘g‘ri», «noto‘g‘ri», «sendvich» variantlarining sezgirligi solishtirilgan. Tomchi usulida antigenni aniqlash uchun virus bilan kasallangan namuna olinib, shirasi chiqarildi va sentrifuga yordamida biroz tiniqlashtirilgandan so‘ng, virusga tayyorlangan

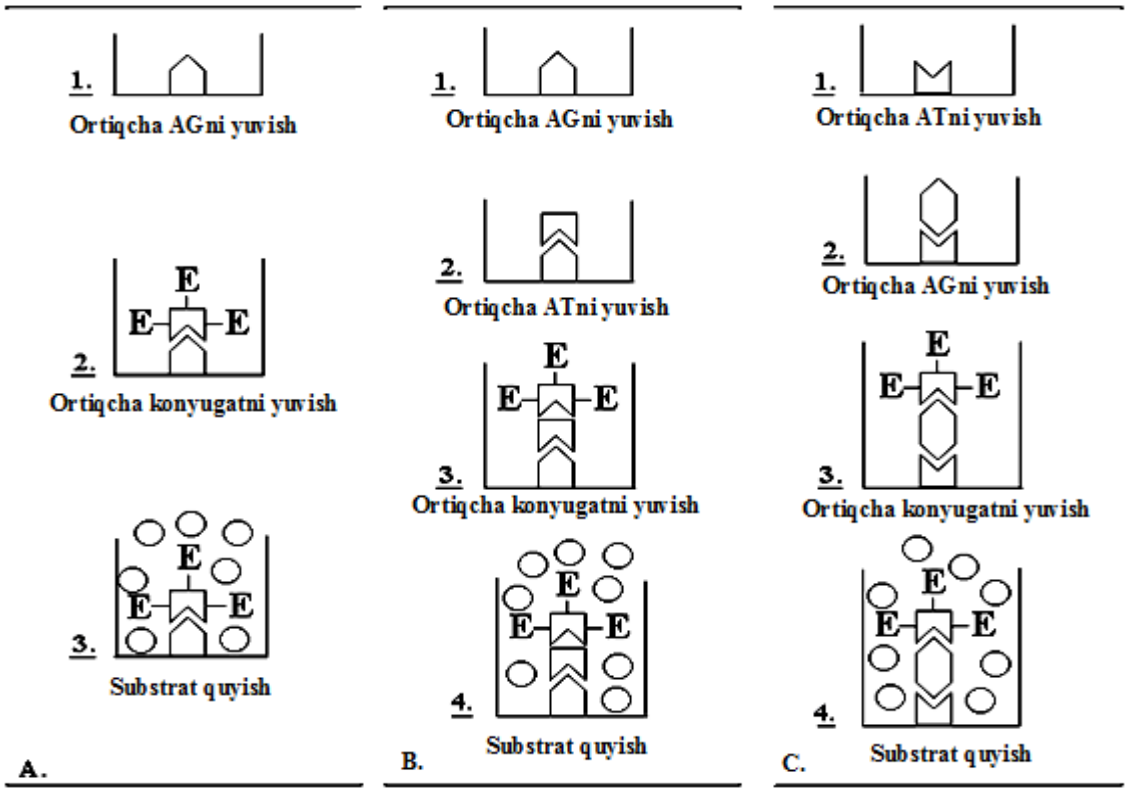
antizardob bilan shisha plastinka yuzasiga teng miqdorda tomizildi va bir necha davomida ko‘zatib borildi, virus mavjud namunada loyqalanish kuzatiladi. Ikkiyoqlama immunodiffuziya usulida virusni aniqlash quyidagicha amalga oshirildi. Virus Uxterloni ishlab chiqqan Zilber va Abelevlar tomonidan mikromodifikatsiya qilingan [10, 13, 16, 17] IID usuli yordamida aniqlandi. Bunda 1% «Difco» agarini 0,1M fosfat buferida (pH 8,0) eritilib, 24 ml 60-65°C sovutilgan suyuq agarni «shayton» (suv tarozi) bilan gorizontal joylashtirilgan, o‘lchami 9×12 sm bo‘lgan shisha plastinka ustiga quyildi. Agar-agar geli qotgandan so‘ng, teshikchalar orasi 4 mm bo‘lgan maxsus shtamplar yordamida yulduzchalar shaklidagi hamda to‘g‘ri chiziq bo‘ylab chuqurchalar tayyorlandi va bu chuqurchalarga 80 mkl dan AG va AZ solingandan so‘ng shisha plastinkalar maxsus moslamaga joylanib, tagiga 200 ml suv solingan eksikatorda 48 soat davomida saqlandi [10, 15, 19, 20]. Bu muddat davomida AG va AT diffuziyasi ro‘y berib, ularning spetsifik to‘qnashishidan pretsipitatsiya liniyalari hosil bo‘ldi. Har bir reaksiya natijalari aks etgan agar bo‘lakchalari yog‘sizlantirilgan buyum shishasi ustiga joylashtirilib, xromotografik yoki filtr qog‘oz bilan yopilgan holatda 20°C da 15-20 soat davomida quritildi. So‘ngra suv tagida namlanib filtr qog‘ozdan tozalandi. Tozalangan agar bo‘lakchalari tarkibida 1% li amidoshvars, metanol, sirka kislotasi va suv (4:4:1) aralashmasidan iborat bo‘lgan maxsus bo‘yoqqa solinib 10 daqiqa davomida pretsipitatsiya chiziqlari bo‘yaldi va undan so‘ng preparat «yuvuvchi» suyuqlik (bo‘yoqsiz metanol, sirka kislotasi va suv) bilan bir necha marta yuvildi. Quritilgandan so‘ng reaksiya natijalari hisobga olindi. IID yordamida tadqiq qilinadigan o‘simlik namunasi quyidagicha tayyorlandi: o‘simlik bargi hovonchada ezilib shirasi chiqarildi va 1:1 nisbatda 0,02M li fosfat buferi (pH 7,5) bilan aralashtirildi, so‘ng 8000 ayl./daq.da 10 daq. davomida sentrufuga qilingandan so‘ng cho‘kma usti suyuqligi (ChUS) olindi. ChUS AG sifatida ishlatildi [3, 18, 22, 23, 24].

NATIJALAR VA TAHLILI

IFA ning «to‘g‘ri» varianti yordamida virusni diagnostika qilish uchun dastlab qattiq fazaga AG 3-4 soat 37°C da immobillandi, ortiqcha AG yuvib tashlanib, ustidan xuddi yuqoridagidek konyugat immobillandi. Ortiqcha konyugat ham yuvib tashlangandan so‘ng polistirol planshetlarning chuqurchalariga substrat solinib reaksiyaning borishi 30-60 daqiqa davomida kuzatib borildi (rasm, A).

«Noto‘g‘ri» variant yordamida virusni diagnostika qilish uchun ham dastlab qattiq fazaga AG 37°C da 3-4 soat davomida immobillangandan so‘ng ortiqcha AG yuvib tashlanib, ustidan AT xuddi yuqoridagidek immobillandi. Ortiqcha AT ham yuvib tashlanib, ustidan kon’yugat 3-4 soat, 37°C da immobillandi, undan so‘ng

ortiqcha kon'yugat yuvib tashlanib substrat solindi va reaksiyaning borishi kuzatib borildi (rasm, B). IFA ning «sendvich» varianti yordamida viruslarni diagnostika qilish uchun esa oldingi ikki variantdan farqli o'laroq dastlab qattiq fazaga AT xuddi yuqoridagidek immobillandi, ortiqcha AT yuvib tashlanib ustidan AG immobillandi va ma'lum vaqtdan so'ng ortiqcha AG ham yuvib tashlandi. So'ngra ustidan kon'yugat solinib 3-4 soat davomida (37°C) immobillanilib ortiqcha konyugat yuvib tashlandi va substrat solinib reaksiyaning borishi 30-60 daqiqa davomida kuzatib borildi (rasm, V).



Shartli belgilar: ⌴ - qattiq faza (polistirol), ⬡ - AG, ✉ -AT, E—E—E - konyugat (IgG + ishqoriy fosfataza), ○ -substrat (p-nitrofenilfosfat), **E**- ferment.

Rasm. Viruslarni IFA ning turli variantlari yordamida daignostika qilishning sxematik ko'rinishi: A-«to'g'ri» variant, undagi 1-AG ni immobillash, 2-konyugatni immobillash, 3-substrat solish; B-«noto'g'ri» variant, undagi 1-AG ni immobillash, 2-AT ni immobillash, 3-konyugatni immobillash, 4-substrat solish; V-«sendvich» varianti, undagi 1-AT ni immobillash, 2-AG ni immobillash, 3-konyugatni immobillash, 4-substrat solish.

NATIJALAR

Bu variantlar bilan bir qatorda IID va TU kabi boshqa immunologik usullar sezgirligi ham solishtirildi. Olingan natijalar jadvalda keltirilgan.

1-jadval

IFA variantlari va boshqa immunologik usullar sezgirligini aniqlash

T.r.	IFA variantlari va usullar	Suyultirish darajasi, marta										
		N	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
		Reaksiya ko'rsatkichlari										
1.	Tomchi usuli	+	+	-	-	-	-	-	-	-	-	-
2.	IID	++++	+++	++	+	+	-	-	-	-	-	-
3.	«notog'ri»	++++	+++	++	+	+	-	-	-	-	-	-
4.	«tog'ri»	++++	++++	+++	+++	++	++	+	+	-	-	-
5.	«sendvich»	++++	++++	+++	+++	++	++	++	+	+	+	-

Izoh: jadvaldagi «+» - reaksiya ko'rsatkichi och sariq rangda, «++» - sariq, «+++» - to'q sariq, «++++» - o'ta to'q sariq rangda, «-» -rang o'zgarmaganligini anglatadi.

Jadvaldan ko'rinib turibdiki KXV dan yuqorida aytib o'tilgandek tayyorlangan virusli namuna IFA ning «to'g'ri» varianti yordamida diagnostika qilinganda, variant namunani 10^{-7} gacha, «noto'g'ri» variantda tekshirilganda esa xuddi shu namunani 10^{-4} gacha, «sendvich» varianti yordamida tekshirilganda esa namunani 10^{-9} gacha aniqlay oldi. Shu bilan bir qatorda KXV dan tayyorlangan namuna tomchi va IID yordamida ham tekshirilib usul sezgirligi taqqoslandi. Tomchi usuli virusni 10^{-1} gacha, IID esa namunani 10^{-4} gacha aniqlay olishi qayd etildi (jadval).

Olingan natijalardan ko'rinib turibdiki (jadval) KXV ni diagnostika qilishda ishlatilgan immunologik usullar ichida eng sezgiri IFA ning «sendvich» varianti ekanligi ma'lum bo'ldi, undan keyingi o'rinda esa IFA ning «to'g'ri» varianti ekanligi va uning sezgirligi 10^{-7} gacha ekanligi ma'lum bo'ldi, ammo bu variantning ham o'ziga xos qulaylik tomonlari ham mavjud. Bunda birinchi navbatda qattiq fazaga AT immobillanmasdan to'g'ridan-to'g'ri AG immobillanadi va ustidan konyugat adsorbsiyalanadi, bu o'z navbatida bir qancha vaqt va AT ning tejalishiga sabab bo'ladi, diagnostika qilish uchun ketadigan umumiy vaqt esa 9 soatu 40 daqiqani tashkil etsa, «sendvich» va «noto'g'ri» variantlari uchun ketadigan vaqt 14 soatni tashkil etadi, ammo «to'g'ri» variantning aniqlik darajasi va sezgirligi «sendvich» varantidan past ekanligi ma'lum bo'ldi. «Noto'g'ri» variant hamda IID usulining sezgirligi bir xil, ya'ni 10^{-4}, Tomchi usulining sezgirligi esa ularning barchasidan past ekanligi ma'lum bo'ldi. A.G.Odinetsning shu yo'nalishda olib borgan tajribalarida VBA, IID va IFA kabi usullarning sezgirlik darajasini solishtirgan va tekshirishda asosan AG sifatida chinnigul mozaika virusidan foydalangan. U yuqorida keltirib o'tilgan usullar yordamida jami 9575 ta namunani tekshirgan. Jumladan VBA usuli yordamida 5045 ta namunani tekshirib, shundan 50%, IID usuli yordamida 2867 ta namunani tekshirib 40%, IFA usuli yordamida esa

1253 ta namunani tekshirib, shundan 58% virus bilan kasallanganligini aniqladi va IFA usulini boshqa immunologik usullardan sezgir ekanligini isbotladi [21, 25, 26].

XULOSA

Olib borgan tadqiqotlarda esa undan farqli o‘laroq, VBA emas balki TU ning, shu bilan bir qatorda IFA ning yuqorida keltirib o‘tilgan uchta variantlarining sezgirligi taqqoslandi va kerakli natijalarga eirishildi. Demak, adabiyotlardan olingan natijalar hamda o‘tkazilgan tajribalar asosida quyidagicha xulosa qilish mumkin: IFA variantlari ichida «sendvich» variantining sezgirligi qolgan tekshirilib taqqoslangan variantlar hamda immunologik usullar ichida eng yuqori ekanligi ma’lum bo‘ldi.

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