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jkbtkLQh;j ekbØkck;ke dh Hkfedk le>u gr eVkVklfØlVkfed –f"Vdk.k% ,d leh{kk

uuk dekjh] mek] ,e-,- bdcky] lkfjdk tk;loky] vfuy jk;] fnu'k dekj

10.18805/BKAP414

lkjk'k

ikèkk dh tM fofoèk l{ethok dk vkJ; nrh g tk ,d nlj vkj ikèkk d lkFk ijLij fØ;k djrh gA jkbtkLQh;j feêh dk {k= g] tk ikèk dh tM l fudVrk l tMk gvk gA bul tM ekbØkck;ke dk jkbtkck;ke dgk tkrk gA bld vè;;u l ikèkk d LokLF; e l{ethok dh Hkfedk d ckj e tkudkjh feyrh gA ,uth,l rduhdk dh çxfr d lkFk] xj&lkL—frd çtkfr;k dh igpku lHko gk xÃ gA ij thukfed Mh-,u-,- ¼eVktukfeDl½ dh vuØe.k vklkuh l VDlkukfed vkj dk;kRed çkQkby dh igpku dj ldrk g yfdu ekbØkck;ke d lfØ; vkj fuf"Ø; lnL;k dh igpku ijh ugÈ gkrh gA eVk&VklfØiV,feDl ij ekbØkfc;y lenk; }kjk 0;ä thu dh igpku djd ,d ekbØkfc;y lenk; d lfØ; dk;kRed çkQkby dk fuèkkfjr dju e enn djrk gA lkekU; dk;çokg ueukdj.k] vkj,u, fu"d"k.k] iLrdky; dh r;kjh] vuØe.k] MVk dh io&çlLdj.k] vlcyh] VDlkukfed@dk;kRed çkQkbçyx vkj vrj vfHk0;fä fo'y"k.k gA ,lh dÃ pukfr;k ektn g] ftUg mUur vuØe.k rduhdk vkj ,Yxkfjne d ekè;e l nj fd;k tk ldrk gA

"kCn dth% to lpukRed fo'y"k.k] eVkVklfØlVkfeDl] ekbØkck;ke] jkbtkLQh;jA

Metatranscriptomic Approach to Understand the Role of the Rhizosphere Microbiome: A Review

Naina Kumari, Uma, M.A. Iquebal, Sarika Jaiswal, Anil Rai, Dinesh Kumar

ABSTRACT

Plant roots harbour diverse microorganisms that interact with each other and the plants. Rhizosphere is the region of soil, closely associated with plant root. The microbiome associated with them is termed as rhizobiome. Its studies give insights into the roles of the microorganisms in plant health. With the advancement of NGS techniques, the identification of the non-cultural species has turned out to be possible. Sequencing of the whole genomic D.N.A. (metagenomics) can easily identify the taxonomic and functional profile but identification of the active and inactive members of the microbiome is not fulfilled. Meta-transcriptomics helps in determining the active functional profile of a microbial community by identifying the genes expressed by the entire microbial community. The general workflow is sampling, RNA extraction, library preparation, sequencing, pre-processing of data, assembly, taxonomic/functional profiling and the differential expression analysis. There exist numerous challenges, that can be overcome through enhanced sequencing technologies and algorithms.

Key words: Bioinformatic analysis, Meta-transcriptomics, Microbiome, Rhizosphere.

enk d ekbØk,Uok;jeV e vjck l{etho gkr gA ik/k ,d lxfBr i;koj.k e jgr g vkj enk e jgu oky bu l{ethok d lkFk ijLij fØ;k djr gA ,lk gh ,d {k= ^^jkbtkLQh;j^^ g tk ik/kk dh tMk l tMk ,d ldh.k {k= g ftle yk[kk l{ethfo;k d fuokl LFkku gkr gA jkbtkLQh;j l tM l{ethok dk lkefgd :i l tM ekbØkck;ke ;k jkbtkck;ke d :i e tkuk tkrk g ¼Moorthy and Balasundaram] 2021½A jkbtkck;ke ik/k dh of) vkj fodkl dk fu;f=r djd ik/kk d LokLF; e

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egRoi.k Hkfedk fuHkkrk gA jkbtkck;ke e lgk;d] gkfudkjd vkj rVLFk nkuk rjg d l{etho 'kkfey gk ldr g vkj o tMk dk mifuof'kr dju d fy, ,d nlj d lkFk çfrLi/kk djr gA blfy,] fdlh Hkh Qly çtkfr d jkbtkck;ke dk v/;;u Qly l/kkj e egRoi.k Hkfedk fuHkkrk gA

ik/kk dh tMk vkj l{ethok d ijLij fØ;k,

ftl rjg l l{etho ik/k l ijLij fØ;k djr g] pkg og ik/k d fy, Qk;nen gk ;k gkfudkjd] o ,d ik/k dh db fo'k"krkvk dk çHkkfor dju d fy, ftEenkj gkr gA ; l{etho ik/k d gkekuy Lrj d lkFk&lkFk feêh l ik"k.k çkIr dju e Hkh egRoi.k Hkfedk fuHkkr g ¼Moorthy and Balasundaram] 2021½A bld vykok] ik/k ik"kd rRok d vo'kk"k.k e vkj fofHkUu tfod vkj vtfod rukok l lj{kk çkIr dju d fy, l{ethfo;k ij Hkh fuHkj djr gA fdlh Hkh çk—frd okrkoj.k e l{ethoh vkerkj ij Lor=:i e ugh jgr gA o i;koj.k e ektn ijikf"k;k d lkFk&lkFk ,d nlj d lkFk Hkh ijLij fØ;k djr g vkj ,d tfVy ç.kkyh dk fuek.k djr gA jkbtkLQh;j ik/k dh tM vkj feêh d chp bVjQl gku d dkj.k l{ethfo;k dh ifjofÙk vkj NkV&NkV {k=k e jgu oky thok dk vkJ; nrk gA u doy l{ethoh ik/kk ij çHkko Mkyr g] cfYd ik/k Hkh mu ij çHkko Mkyr gA ik/kk dh tM dkcu&le) lCIVV dk lko djrh g tk vfLFkj lCIVV gkr g vkj lHkor% l{ethfo;k d i{k/kj gkr g rFkk mUg tYnh l vkRelkr dj ldr g ¼Kumar and Dubey] 2020½A tM d vo;o tl dkckgkbMV] dkcfud ,flM vehuk ,flM vkj ldUMjh eVkckykbVl Hkh tMk dh vkj çjd cDVhfj;k dk vkdf"kr dj ldr g ¼Carvalhais *et al*] 2013½A bl çdkj] ik/k vkj l{ethfo;k d chp f}rjQk ijLij fØ;k, gkrh g] tk ;k rk ,d nlj dh of) vkj fodkl d i{k e ;k fQj of) vkj fodkl dk ckf/kr dju okyh gkrh gA

jkbtkLQh;j ekbØkck;ke v/;;u eVkVklfØlVkfeDl dh Hkfedk

ekbØkck;ke dk le>u d fy, ikjifjd lo/ku fof/k;k jkbtkck;ke d v/;;u dk ckf/kr djrh gA ; fof/k;k doy 1% cDVhfj;k dh igpku dj ldrh g] tk ey :i l —f"k ;kX; gh gkr gA xj&—f"k ;kX; vkj v;kX; l{ethok dk ikjifjd rjhdk l v/;;u djuk yxHkx vlHko gA fiNy dN n'kdk e lhDoflx çk|kfxfd;k e çxfr u tfod ç;kxk d rjhd dk cny fn;k g] [kkldj tc ekbØkck;ke d v/;;u dh ckr vkrh gA eVkthukfed fof/k dk çe[k nk"k ;g g fd ;g ekbØkck;ke d lfØ; vkj fuf"Ø; lnL;k d chp vrj dju e vleFk gA bld vykok] o mu lnL;k d chp vrj dju e Hkh vleFk g tk okLro e ml le; okrkoj.k e ;kxnku n jg g vkj tk fcuk ;kxnku d flQ ektn gA ,d ekbØkfc;y lenk; viuh cnyrh i;koj.kh; ifjfLFkfr;k d çfr dl çfrfØ;k djrh g] bldh lEi.k tkudkjh çkIr dju d fy, cMh ieku ij eVkVklfØlVkfeDl dk;'kyh dk bLreky fd;k tk ldrk g ¼Shakya *et al*.] 2019½A ,d liy dk eVkVklfØlVke ml liy e fdlh fn, x, {k.k e vkj fof'k"V i;koj.kh; ifjfLFkfr;k e dy mRNA dk dlpj dju oky thu d ckj e ,d fopkj nrk gA bl çdkj] ij ekbØkfc;y dE;fuVh }kjk dku l thu 0;ä fd, tk jg g] bldh igpku djd eVkVklfØlVkfeDl ,d ekbØkfc;y dE;fuVh d lfØ; QD'kuy çkQkby dk fu/kkfjr dju e enn dj ldrk g ¼Aguiar and Pulido *et al*-] 2016½A ik/kk&l{ethoh dh ijLij fØ;k d v/;;u lfgr eVkVklfØlVkfeDl d fofHkUu vuç;kx g D;kfd ;g fof'k"V dk;k d fy, ftEenkj ekbØkck;ke lnL;k dk fpfàr dju vkj ijik"kh&ekbØkck;ke lc/k d fy, ftEenkj thu dh igpku dju e l{ke gA vr% jkbtkLQh;j ekbØkck;ke v/;;uk e eVkVklfØlVkfed dh Hkfedk cgr egRoi.k gA

eVkVklfØlVkfeDl v/;;u dh lkekU; dk;çokg

fdlh Hkh eVkVklfØlVkfed v/;;u dh lkekU; dk;çokg e fuEufyf[kr pj.k 'kkfey g% ¼i½ liy lxg] ¼ii½ dy RNA fu"d"k.k] ¼iii½ ykbcjh&çcU/k vkj lhDoflx] ¼iv½ eVkVklfØlVkfed MVk dk tolpuk çlLdj.k vkj ¼v½ fMQjf'k;y ,Dlç'ku fo'y"k.kA liy lxg d ckn] ble l dy RNA fudkyk tkrk g vkj fdlh Hkh DNA ln"k.k dk nj dju d fy, DNase fu:i.k fd;k tkrk gA fQj ;kX; RNA dk VdM dh tkp vkj x.koÙkk ijh{k.k d fy, Hktk tkrk gA bld ckn] liy mRNA lEiUuhdj.k d fy, Hktk tkrk g tk ,d egRoi.k vkj ef'dy dk; gA mRNA lEiUuhdj.k d fy, fofHkUu rduhdh;k dk viuk;k x;k g] tl fd 16 ,l vkj 23 ,l vkj-vkj-,u-,- tkp ldj.k ;k vkj-vkj-,u-,- dlpj flLVe ;k 5′&3′ ,DlkU;fDyt ,tkbe d ek/;e l rRNA vkj tRNA dk

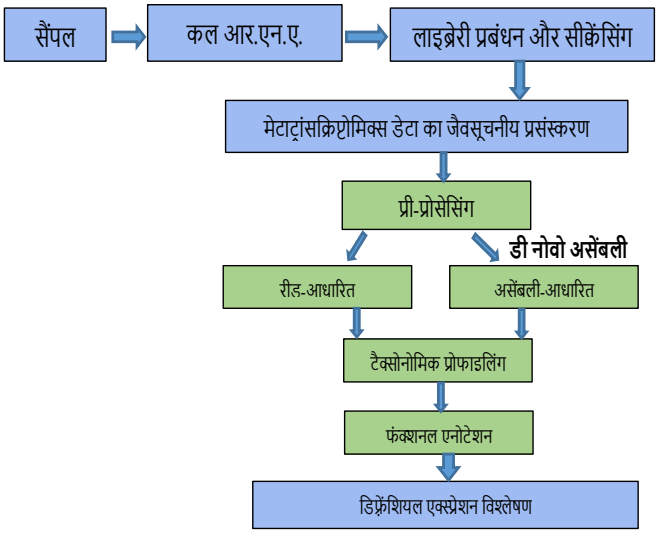
fMxM'kuA mRNA lEiUuhdj.k d ckn] cDNA ykbcjh& çcU/k dh tkrh g vkj lhDoflx d fy, liy Hkt tkr gA bld ckn eVkVklfØlVkfed MVk dk fo'y"k.k fd;k tkrk g ftldh foLrr ppk uhp fn, x, vuHkkx e dh xb gA dk;çokg e vfre pj.k] eVkVklfØlVkfed MVk dk fMQjf'k;y ,Dlç'ku fo'y"k.k gA ble fofHkUu i;koj.kh; ifjfLFkfr;k vkj ekinMk e ekbØkck;ke dh ryuk djuk vkj le; d lkFk ekbØkfc;y tojklk;fud QD'ku ij mud çHkko dh ryuk djuk 'kkfey g ¼fp= 1½A fMQjf'k;y ,Dlç'ku fo'y"k.k d fy, db VYl fodflr fd, x, g tk vkerkj ij çfr liy vkj çfr thu/ VklfØlV cgrk;r e buiV MVk yr gA DeSeq2, Limma, edgeR vkj Cufflinks tl VYl bl mí'; d fy, vDlj mi;kx fd, tku oky VYl gA GAGE tl VYl dk mi;kx ;g igpkuu d fy, fd;k tk ldrk g fd dku l ikFkot ,d fLFkfr e nljh fLFkfr l cgrj gA

eVkVklfØlVkfeDl MVk dk tolpuh; çlLdj.k çh&çklflx

v'k)rk dk de dju d fy, MkmuLVhe fo'y"k.k l igy v'k) jhM dk gVku ;k fVe dju d fy, igyk vkj lcl egRoi.k dne MVk dh x.koÙkk fu;=.k gA dN x.koÙkk fu;=.k ekunMk ftudk vulj.k fd;k tkrk g¼i½ fuEu&x.koÙkk oky Vfeuy cl dk gVkuk ¼ii½ 25 l uhp d vklr x.koÙkk Ldkj oky jhMl dk gVkuk ¼iii½ lhDol l vLi"V cl dk gVkuk vkj ¼iv½ de yckb d oky jhMl dk gVkukA liy l vR;f/kd çpj ek=k e rRNA VklfØlVl dk gVkuk ,d egRoi.k dne g ftl /;ku e j[kk tkuk pkfg, D;kfd ; VklfØlVl MkmuLVhe fo'y"k.k e leL;k ink djr gA ijik"kh tho d jhMl dk gVkuk] tl fd ekuo ekbØkck;ke liy l ekuo jhMl dk gVkuk] db eVkVklfØlVkfed v/;;u dh vko';drk gk ldrh g tk ikjifjd jhM efix fof/k;k tl fd v/;;u d rgr VkjxV ijik"kh d lnHk thuke dk ei djd fd;k tk ldrk gA

Mh ukok vlcyh

mPp x.koÙkk oky fQYVj fd, x, jhMl dk Mh ukok vlcyh }kjk l;VfVo VklfØlVl e bdëk fd;k tk ldrk gA pfd fofHkUu l{ethok d fy, lnHk thuke vuiyC/k g] blfy, Mh ukok vlcyh ,d lnHk vk/kkj miyC/k djkrh g tk yc] ,DlçLM thuke lxeV dk çfrfuf/kRo djrk g vkj tk thu dk ,d lnHk lV çnku dj ldrk gA ;g mi;kxdrkvk dk vf/kd ljy rjhd l gkeky,x [kktu dh {kerk çnku djrk g] VDlkukfed ey LFkkfir djrk g] vkj ,DLç'ku fo'y"k.k d lkeu efix d fy, ,d lnHk d :i e dk; djrk g ¼Shakya *et al.*] 2019½A vkt Hkh] eVkthuke vlcyh d fy, mi;kx fd, tku oky VYl tl fd oYoV ¼Zerbino and Birney 2008½ fVfuVh ¼Grabherr *et al*-] 2011½ exkfgV ¼Li *et al*-] 2015½ vkj eVkLiMl ¼Nurk *et al*-] 2017½ eVkVklfØlVkfed vlcyh d fy, mi;kx fd, tkr gA yfdu bu VYl d ifj.kke lVhd ugh gk ldr gA VklfØlV vlcyh xkQ ¼Vh-,-th½ vkj vkbMhch,&,eVhih eVkVklfØlVkfed jhMl vlcyh d fy, dN fof'k"V VYl gA



fp= 1¼ eVkVklfØlVkfeDl v/;;u dh lkekU; dk; çxfrA

VkIfØIV VDIku,eh

,d ekbØkck;ke liy dh dE;fuVh ljpuk dh igpku ;g tkuu d fy, fd mue cgrk;r d lkFk D;k ektn g] fdlh Hkh ekbØkck;ke v/;;u e lcl egRoi.k dne g ftl VDlkukfed çkQkbfyx ;k VkIfØIV VDlku,eh dgk tkrk gA RNA liy e dku l tho lfØ; :i l ,Dlçl gk jg g] ;g le>u d fy, VDlkukfed vlkbueV dk jhM&vk/kkfjr ;k d,fUVx&vk/kkfjr fd;k tk ldrk g ¼Shakya *et al.*] 2019½A VDlkukfed çkQkbfyx dju d fy, vyx&vyx rjhd g tl fd VkIfØIVl dh igpku vkj oxhdj.k d fy, vk-Vh-;- dk mi;kxA bl rjhd e vkerkj ij 16S ;k 18S rRNA lhDol fudkyu d fy, lhDol l,fVx vkj fQj oxhdj.k d fy, ekbØkck;ke MVkcl e efix 'kkfey gA pfd eVkVkIfØIVkfed MVk e vkj-vkj-,u-,- lhDol 50% l de g vkj vDlj gVk fn;k tkrk g] ;g rjhdk lkf[;dh; :i l 0;ogk; ugh g] yfdu igy VDlkukfed çkQkby dh igpku dju d fy, vkj-vkj-,u-,- lhDofll dk cuk, j[kk tk ldrk gA fQj mUg vkx d MkmuLVhe fo'y"k.k d fy, gVk fn;k tk ldrk gA vU; rjhdk] ekdj thu oky MVkcl d lkFk vykbu dju d fy, VDlkukfed vlkbueV d fy, li.k lhDofll dk mi;kx djuk g ¼Jagtap *et al*] 2021½A ekbØkck;ke v/;;u e QkYl i,ftfVo fgV l lhDol fgV dh de l[;k dk vrj ,d cMh leL;k ink djrk gA bld vykok] ekbØkfc;y fofo/krk ij lhfer Kku Hkh ekbØkck;ke d eVkVkIfØIVkfeDl v/;;u e VDlku,eh oxhdj.k VYl d vuç;kx dk xHkhj :i l lhfer djrk g ¼Ranjan *et al*] 2021½A

QD'kuy ,ukV'ku

fdlh Hkh eVkVkIfØIVkfed v/;;u d ykHkk e l ,d g] ml thu rd igp] tk liy e ekbØkfc;y dE;fuVh }kjk 0;ä dh tkrh gA mud }kjk fuHkkb xb QD'kuy Hkfedk dk le>u d fy, bu thuk dh igpku dh tk ldrh gA D;kfd ,DlçLM VkIfØIVl okLrfod QhukVkbi d fy, ,d ç,Dlh dk çfrfuf/kRo djr gA VkIfØIVl d QD'ku dk fpfàr djuk eVkVkIfØIVkfeDl d fy, ,d egRoi.k dk; g ¼Shakya *et al.*] 2019½A QD'kuy ,ukV'ku jhM&vk/kkfjr ;k vlcy fd, x, d,fUVx l Hkh gk ldrk gA MetaCLADE, HMM&GRASPx vkj UProC tl VYl jhM&vk/kkfjr QD'kuy ,ukV'ku djr g] ftUg vkerkj ij buiV d :i e vuekfur vkiu jhfMx Ýe ¼ORF½ dh vko';drk gkrh gA FragGeneScan vkj Prodigal tl thu [kkt dk;Øek dk mi;kx vlcyh vk/kkfjr QD'kuy ,ukV'ku d fy, fd;k tk ldrk g ¼Shakya *et al.*, 2019½A feuikFk ;k vkbikFk tl eVkckfyd ikFkot dh efix }kjk QD'kuy ,ukV'ku çkIr dju d ckn VkIfØIVl d ,tkbefVd QD'kUl dk Hkh çkIr fd;k tk ldrk gA

jkbtkLQh;j eVkVkIfØIVkfeDl dk;'kyh dh pukfr;k

¼i½ 'k,V&mRNA gkQ&ykbQ

mRNA e dN ldM l dN feuVk dh lhek d lkFk cgr NkVh gkQ&ykbQ gkrh g] blfy, ikfjfLFkfrd míhiu d fy, rth l/vYidkfyd ldrk dk ukfVl djuk ef'dy gkrk gA blfy,] lEiYl dk &80 fMxh lfYl;l ;k vkj-,u-,- ifjj{k.k fofy;u e lxfgr fd;k tkuk pkfg,A

¼ii½ enk d liy l RNA dk vkblky'ku

vi;kIr ly&yk;fll] enk e RNA v.kvk dk vf/k'kk"k.k vkj enk e RNases tl vojk/kd ,tkbek dh mifLFkfr d dkj.k enk liy l RNA dk vkblky'ku ,d ef'dy dke cu tkrk gA

¼iii½ mRNA lo/ku

dy RNA e e[; :i l rRNA vkj tRNA 'kkfey gkr g yfdu mRNA doy 1&5% gkrk g] blfy, mRNA lo/ku d fy, fofHkUu rduhfd;k dk fu;kftr dju dh vko';drk gkrh gA bld vykok] rRNA dk gVkuk Hkh mRNA lo/ku d nkjku fopkj fd;k tku okyk ,d egRoi.k dkjd g D;kfd rRNA fdlh Hkh eVkVkIfØIVkfeDl MVk dk yxHkx 90% fgLlk gkrk gA gkykfd bu rRNA dk vDlj lhDol l igy vk.kfod vçkp dk mi;kx djd gVk fn;k tkrk g] yfdu lifyl e mud çHkRo d ifj.kkeLo:i rRNA dh dN ek=k rc Hkh lhDoULM gkrh g ftl MVk fo'y"k.k d le; fQYVj dju dh vko';drk gkrh g D;kfd ;vuko';d :i l MVk dh ek=k dk c<kr g vkj ikFkot o.ku ;k fM¶jf'k;y ,DLç'ku fo'y"k.k e dkb ;kxnku ugh djr gA

¼iv½ cDNA l'y"k.k

cDNA LVM d fjol VkIfØl'ku d nkjku =fV;k vk ldrh gA bld vfrfjä] ;fn VElyVe ,d nlj d fy, mPp Lrj dh le:irk gk rk dkbefjd cDNA v.k mRiUu gk ldr gA gkykfd] bl leL;k dk lh/k RNA lhDoflx }kjk Vkyk tk ldrk g] yfdu RNA {kj.k dk jkdu d fy, mi;ä mik; fd, tku dh vko';drk gA

¼v½ eVkVk lfØlVkfed MVk dh vlcyh

d,fUVx e eVkVk lfØlVkfed 'k,V jhMl dh vlcyh ,d vfuok; fo'y"k.kRed dne ugh gA ;fn dkb jhMl dk vlcy djuk pkgrk g] rk ml mPpx.koÙkk oky ç;kxkRed lhDol MVk dh vko';drk gkrh g ftle jhMl dh xgu lhDoflx gkrh gA

¼vi½ eVkVk lfØlVkfeDl MVk dk mi;kx djd tfod rF;k dh 0;k[;k

RNA-seq MVk vkj li.k DNA MVk nkuk d fo'y"k.k dk bVhxfVx djuk eVkVk lfØlVkfeDl MVk d fo'y"k.k vkj 0;k[;k e ,d pukrhi.k dk; gA ,d liy d fy, ,d gh le; e bu nk MVk çdkjk dk eY;kdu dju dh vR;f/kd vu'kl dh tkrh g rkfd i.k ,DlçLM thu cuke lHkkfor ekrnk thu d chp ryukRed v/;;u fd;k tk ldA

fu"d"k

fof'k"V i;koj.kh; ifjfLFkfr;k e ,d fof'k"V le; ij ,d ekbØkck;ke d ekbØkfc;y VklfØlV dk ijk Kku eVkVk lfØlVkfeDl v/;;u d ek/;e l lVhd :i l çkIr fd;k tk ldrk gA enk eVkVk lfØlVkfeDl d 0;kid Kku dk mi;kx feêh d ik"kd rRo lo/ku vkj l?kVu] jkxtud l{ethfo;k dk gVku] ik/kk dh of) dk c<ku vkj tfod vif'k"V inkFkk vkj çn"kdk d iupØ.k dh lEi.k le> çkIr dju e fd;k tk ldrk gA blfy,] ekbØkfc;y eVkVk lfØlVkfeDl dk ,d vPNk Kku LFkyt bdkfLVe l fuiVu d fy, çHkkoh rduhfd;k d fodkl e lgk;rk dj ldrk g ¼Ranjan *et al*-] 2021½A ;|fi eVkVk lfØlVkfed MVk d eY;kdu vkj 0;k[;k e db pukfr;k g ijr i;koj.kh; mRNA l eVkVk lfØlVke MVkcl dk fuek.k dju dh pukfr;k cgr ljy gA ;g tfod rF;k dk [kktu dk ,d çHkko'kkyh vkj mfpr rjhdk j[krk g ftl fofHkUu thukfed i)fr;k d ek/;e l Nik;k tk ldrk gA gj ub rduhd fiNyh dfe;k dk ijk djr g, curh gA lkotfud Mkeu e tek fd, x, eVkVk lfØlVkfeDl MVk egRoi.k thok] thuk vkj ikFkot dh [kkt d fy, Hkfo"; e cM MVk fo'y"k.k vkj of'od eVk&fo'y"k.k d fy, dke vkr gA ji,flVjh Hkh ekbØkck;ke MVk d cg&vkfeDl fo'y"k.k d fy, dke vkr g tk gky gh e fofHkUu i;koj.kh; ifjfLFkfr;k e ekbØkck;ke dh Hkfedk dk le>u d fy, egRoi.k lkfcr gk jg gA

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