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September 15, 2005

Dr. Yves Brun
Systems Biology Faculty Search
Department of Biology
Indiana University
Jordan Hall 142, 1001 E 3rd Street
Bloomington, IN 47405-7005

Dear Dr. Brun,

I am writing to provide a letter of reference for Dr. Xuelu Wang, who has applied for a position you recently advertised. Xuelu was a graduate student in my laboratory. He began working in the fall of 1995, and he completed his Ph.D. in the fall of 2000. Afterward, he continued on as a postdoctoral research associate for a short time, working on several aspects of his dissertation research.

Xuelu did several rotations in my department before deciding to do his Ph.D. research in my laboratory. I think he made his decision to work with me based on his background in wheat breeding and seed protein analysis. At the time Xuelu started, we had recently discovered a relationship between the concentration of elongation factor 1α (eEF1A) and the lysine content of maize endosperm. The *opaque2* (*o2*) mutation, which typically leads to a higher endosperm lysine content, causes a two- to three-fold increase in the level of eEF1A. The increase in lysine occurs because *o2* reduces the synthesis of zein storage proteins, which contain no lysine, while it increases the accumulation of lysine-containing proteins, as well as free lysine. We found a high correlation ($r = 0.9$) between the concentration of eEF1A and the total lysine content of endosperm flour for most maize inbreds. Only in one (Oh545*o2*) was there a poor correlation, and we found that about one third of the lysine in Oh545*o2* is free lysine; furthermore, this inbred has an exceptionally high level of free amino acids (FAA) in general. These observations provided the foundation for Xuelu's dissertation project, which was directed at answering two fundamental questions: 1) How many loci influence the level of eEF1A in maize endosperm, and can eEF1A be used as a selectable marker for increasing the lysine content of maize endosperm? 2) What is the basis for the exceptionally high level of free lysine and FAA in Oh545*o2*.

Xuelu set up a genetic experiment to approach these questions. He created a segregating F2 population from a cross between Oh51A*o2* (high eEF1A and low free lysine) and Oh545*o2* (low eEF1A and high free lysine); the F3 ears were used for phenotyping the level of eEF1A and FAA and DNA from F2 leaves was used for genotyping this population. The F2 showed continuous segregation for eEF1A content, but somewhat discontinuous segregation for FAA content. Xuelu created an SSR-based linkage map for the parents and used it for a QTL analysis of the progeny. With regard to eEF1A segregation, he identified two QTLs that explained about 25% of the phenotypic variation. Both of these loci are associated with zein storage protein genes. Since zeins contain no lysine, he sought an explanation for this unexpected correlation. He used an ELISA to measure the amount of the zeins associated with these two loci and found a marked difference between the parents. The low eEF1A parent has 10-fold less α-zein, the major filler protein in protein bodies. With the help of a postdoc, Xuelu isolated protein bodies from the parental inbreds and estimated their average volume. The results showed that the high eEF1A parent had protein bodies with 40% less volume than the low eEF1A parent. Since eEF1A appears to be associated with a cytoskeletal network around the protein bodies, he hypothesized that the higher level of eEF1A (hence protein-bound lysine) is associated with a more extensive cytoskeleton in Oh51A*o2*. Another graduate student recently tested this hypothesis using a set of recombinant inbred lines that were developed from Xuelu's original cross, and his results were supportive. Xuelu did some mRNA profiling experiments with the high and low eEF1A F3

progeny using Curagen's gene calling technology (this was done in collaboration with scientists at Pioneer Hi-Bred), but the data turned out to be too complex to evaluate.

Xuelu identified four QTLs associated with the high FAA phenotype of Oh545*o2*. One of these coincided with the map location of several genes involved in the aspartate pathway: aspartate kinase and homoserine dehydrogenase. To evaluate this relationship, he assayed most of the key enzymes of the aspartate pathway, as well as lysine degradation. These experiments showed that the only significant enzymatic difference between the two parents was lysine feedback inhibition of aspartate kinase. This result led him to identify a monofunctional aspartate kinase gene in the arabidopsis EST database. He used this clone to identify maize homologues and ultimately obtain several full length cDNA clones. These sequences were mapped to determine if they corresponded to the same locus as his QTL. One of the cDNAs did, and he used it as a probe to clone the corresponding alleles from the parental inbreds. Analyses of these gene sequences showed there is one amino acid variation that appears to explain the different feedback inhibition properties of the enzymes.

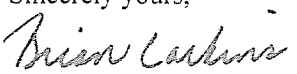
As you can see, Xuelu's dissertation research covered a broad range of genetic, molecular genetic and biochemical questions, and he learned a variety of techniques and concepts as a consequence. While he started with a good background in genetics and breeding, he gained a great deal of experience in molecular approaches. We published his initial findings in three manuscripts in Plant Physiology, and we will soon publish an additional manuscript describing the monofunctional aspartate kinase genes and their expression in maize endosperm. In this regard, I should mention that Xuelu wrote a draft of the three Plant Physiology papers, and I polished them into final manuscripts. The first paper he wrote was not very good, but by the time he got to the third manuscript, he had learned how to write a paper and his ability to do so improved greatly.

Xuelu has a number of traits that will make him a good researcher. He is self-motivated and a hard worker. Once he understands the nature of a research question, he is able to go to the literature for additional background information and continue working with a minimum of direction. He is not shy about presenting his results and defending them to his colleagues. I believe Xuelu has acquired a very good set of technical and computer skills that will allow him to pursue a variety of research questions related to plant genetics and biochemistry.

Xuelu's use and enunciation of English was poor when he started his graduate work. In fact, he was just plain difficult to understand. Because we require our graduate students to TA for at least one semester, it was necessary for Xuelu to enroll in several English courses to improve his speaking. He put a great deal of effort into this, and he also voluntarily enrolled in a course taught by the College of Education where students are videotaped while teaching a class they prepare. As a consequence of these training experiences, Xuelu's ability to speak and be understood has improved immensely. I would add that the time and effort Xuelu put into improving his ability to communicate in English is commensurate with the degree of motivation he brings to his research projects.

I cannot comment on the details of the research Xuelu has been doing in Joanne Chory's lab at the Salk Institute; however, I'm sure you will hear from Joanne regarding Xuelu's accomplishments there. I am confident that Xuelu will be a conscientious and hard working faculty member. If you have any questions about his research experience or personality that I have not addressed, please don't hesitate to give me a call.

Sincerely yours,



Brian Larkins
Porterfield Professor of Plant Sciences

THE SALK INSTITUTE

September 17, 2005

Dr. Yves Brun.
Systems Biology Faculty Search.
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Indiana University.
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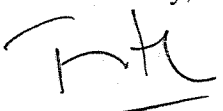
Dear Dr. Brun,

I am writing to recommend Xuelu Wang for a faculty position in the Department of Biology at Indiana University. I know Xuelu through a collaboration we have had with Joanne Chory's group on signaling by the brassinosteroid (BR) LRR-receptor-like kinase (RLK), BRI-1 in Arabidopsis. The BRI-1 project turned out to be very successful, and Xuelu's finding that BRI-1 is constitutively dimerized in the absence of BR ligand, and that its kinase activity is negatively regulated by the C-terminal tail is a novel principle in receptor kinase regulation. A paper describing these studies was recently published in Developmental Cell. Xuelu now has a new unpublished story where he has identified a BRI-1 interacting protein, BKI-1, that acts as an inhibitor of BRI-1 in vitro, and dampens BRI-1 signaling when overexpressed in plants. Conversely, loss of function mutations in BKI-1 result in sensitization of BRI-1 signaling in the plant. BKI-1 is localized to the plasma membrane, even in the absence of BRI-1, but translocates away from the membrane when cells are stimulated by BR, suggesting that dissociation of BKI-1 from BRI-1, possibly as a result of BIR-1-mediated phosphorylation of BKI-1, is an important step in BRI-1 activation of downstream signaling. Again, this seems likely to be a new principle for receptor kinase regulation, and his work on BKI-1 is at a very exciting stage.

Although I have not interacted with Xuelu on a daily basis, I have spent a fair amount of time discussing his data and talking about what experiments to do next. Xuelu is clearly smart, and works very hard. He had strong training in QTL genetics in maize with Brian Larkins and obviously has had an outstanding training in Arabidopsis cell biology and genetics with Joanne. There are sill many gaps in our understanding of BRI-1 signaling, and Xuelu's future research plans to elucidate additional steps in BRI-1 activation, signaling to the nucleus and desensitization, and in particular the role of BKI-1 in regulating BRI-1, are well thought out and seem likely to be informative. His long term plan to extend his studies to an investigation of the functions of other families of LRR RLKs makes sense given his expertise, and should shed light on the currently rather mysterious functions of this very large family of receptor. All in all, I think Xuelu stands a very good chance of being successful as an academic scientist. In summary, I recommend Xuelu very strongly for this position.

Yours sincerely,



Tony Hunter
American Cancer Society Research Professor

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